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THE
JOURNAL OF
EXPERIMENTAL MEDICINE

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NEW YORK
D. APPLETON AND COMPANY
LONDON : 33 BEDFORD STREET, COVENT GARDEN
1900-1901

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CONTENTS

No. 1. OCTOBER 1, 1900.

PAGE

- I. MALLORY, F. B. Proliferation and Phagocytosis. . . 1
- II. MALLORY, F. B. A Contribution to Staining Methods.
1. A Differential Stain for Connective-Tissue Fibril-
læ and Reticulum. 2. Chloride of Iron Hæma-
toxylin for Nuclei and Fibrin. 3. Phosphotungstic
Acid Hæmatoxylin for Neuroglia Fibres. 15
- III. PATON, STEWART. A Study of the Neurofibrils in the
Ganglion Cells of the Cerebral Cortex. 21
Plate I.
- IV. BALDWIN, HELEN. An Experimental Study of Oxa-
luria, with Special Reference to its Fermentative
Origin 27
- V. HISS, PHILIP HANSON, and ATKINSON, JAMES P.
Serum-Globulin and Diphtheric Antitoxin.—A Com-
parative Study of the Amount of Globulin in Normal
and Antitoxic Sera, and the Relation of the Globu-
lins to the Antitoxic Bodies 47
- VI. ATKINSON, JAMES P. The Fractional Precipitation of
the Globulin and Albumin of Normal Horse's Serum
and Diphtheria Antitoxic Serum, and the Antitoxic
Strength of the Precipitates 67
- VII. HEKTOEN, LUDVIG, and PERKINS, C. F. Refractory
Subcutaneous Abscesses caused by Sporothrix
Schenckii. A New Pathogenic Fungus 77
Plates II and III.

- VIII. SPILLER, WILLIAM G. Pathological Report on a Case of Dermatitis Vesiculo-Bullosa et Gangrænosa Mutilans Manuum (Dühring), with a Consideration of the Relations of Vascular and Nervous Changes to Spontaneous Gangrene and Raynaud's Disease 91
Plates IV and V.

No. 2. OCTOBER 25, 1900.

- IX. OPHÜLS, W. Amyloid Substance and Amylaceous Bodies in Multiple Syphilitic Tumors of the Bones, with Remarks on the Relation of Amylaceous Bodies to Amyloid Substance 111
Plates VI and VII.
- X. HAMILTON, ALICE. On the Presence of New Elastic Fibres in Tumors 131
Plates VIII and IX.
- XI. HOWARD, W. T., JR. A Case of General Gaseous Emphysema with Gas Cysts in the Brain formed after Death and due to *Bacillus mucosus capsulatus*, with a Consideration of the Gas-producing Properties of Certain Members of this Group in the Cadavers of Animals 139
Plate X.
- XII. NORRIS, CHARLES, and LARKIN, JOHN H. Two Cases of Necrotic Broncho-Pneumonia with *Streptothrix* . 155
Plates XI-XVI.
- XIII. BURR, CHARLES W., and MCCARTHY, D. J. Acute Internal Hydrocephalus. A Clinical and Pathological Study 195
Plates XVII and XVIII.
- XIV. COWIE, D. MURRAY. A Preliminary Report on Acid-resisting Bacilli, with Special Reference to their Occurrence in the Lower Animals 205

No. 3. DECEMBER 15, 1900.

- XV. REED, WALTER, and CARROLL, JAMES. A Comparative Study of the Biological Characters and Pathogenesis of *Bacillus X* (Sternberg), *Bacillus icteroides* (Sanarelli), and the Hog-Cholera *Bacillus* (Salmon and Smith) 215
Plate XIX.
- XVI. JORDAN, EDWIN OAKES. Some Observations upon the Bacterial Self-Purification of Streams 271
Plate XX.
- XVII. DRESBACH, MELVIN. On the Toxicity of Normal Urine 315

No. 4. JANUARY 15, 1901.

- XVIII. HEWLETT, ALBION WALTER. The Superficial Glands of the Oesophagus 319
Plate XXI.
- XIX. FISCHER, MARTIN H. False Diverticula of the Intestine 333
Plates XXII-XXVI.
- XX. DURHAM, HERBERT E. Some Theoretical Considerations upon the Nature of Agglutinins together with Further Observations upon *Bacillus typhi abdominalis*, *Bacillus enteritidis*, *Bacillus coli communis*, *Bacillus lactis aërogenes*, and some other *Bacilli* of Allied Character 353
- XXI. PERKINS, R. G. Report of a Laboratory Epizootic among Guinea-Pigs, associated with Gaseous Emphysema of the Liver, Spleen, and Kidneys, due to *Bacillus mucosus capsulatus* 389
- XXII. OPIE, EUGENE L. On the Relation of Chronic Interstitial Pancreatitis to the Islands of Langerhans and to Diabetes Mellitus 397
Plates XXVII and XXVIII.

No. 5. MARCH 25, 1901.

- XXIII. EWING, JAMES. Malarial Parasitology 429
Plates XXIX-XXXII.
- XXIV. SHILER, CHR. The Nerves of the Capillaries, with Remarks on Nerve-Endings in Muscle. A New Theory of Lymph-Formation and of Glandular Secretion . 493
- XXV. JOSLIN, ELLIOTT P. The Influence of Bile on Metabolism 513
- XXVI. OPIE, EUGENE L. The Relation of Diabetes Mellitus to Lesions of the Pancreas. Hyaline Degeneration of the Islands of Langerhans 527
Plate XXXIII.

No. 6. OCTOBER 1, 1901.

- XXVII. HUTCHISON, ROBERT, and MACLEOD, J. J. R. Alloxuric Excretion in a Case of Leucopenia 541
- XXVIII. BAILEY, FREDERICK RANDOLPH. Studies on the Morphology of Ganglion Cells in the Rabbit. 1. The Normal Nerve Cells. 2. Changes in the Nerve Cells in Rabies 549
Plates XXXIV-XXXVIII.
- XXIX. HERTER, C. A. The Acid Intoxication of Diabetes in its Relation to Prognosis 617
- XXX. McCRAE, JOHN. Notes upon the Agglutinations Obtained by Intraperitoneal Insertion of Celloidin Capsules containing Bacilli and upon a Mode of Preparing Such Capsules 635
- XXXI. MELTZER, S. J. Some Experimental Data on the Significance of Concentration and of Multiplicity of Area in Hypodermic Injections 643
- XXXII. MENDEL, LAFAYETTE B., and HOOKER, DONALD R. Observations on a Case of Cyclic Albuminuria 647
- INDEX 655

PROLIFERATION AND PHAGOCYTOSIS.

By F. B. MALLORY, M. D.

(From the Sears Pathological Laboratory, Harvard University Medical School.)

The effects which injurious agents, especially the toxins secreted by bacteria, produce on tissues are manifested in four different ways:

- 1, by degeneration or necrosis of cells;
- 2, by exudation from the blood-vessels;
- 3, by proliferation of cells; and
- 4, by phagocytosis, this term being used here to mean the inclusion and digestion of certain cells by other cells.

These four processes may occur separately, or in various combinations and proportions. The first two processes are generally accepted and taught. It is with reference to the last two, proliferation and phagocytosis, that I wish to present a certain amount of evidence bearing on the character of the cells concerned, on the nature of the toxins which excite the processes, on the duration and termination of the lesions, and on the relation of the processes to repair.

The view that toxins can cause various cells to proliferate, and certain cells to become phagocytic for other cells, was first forcibly called to my attention by a histological study of the lesions of typhoid fever published in this JOURNAL in 1898.¹ Since then I have studied these processes in various diseases in nearly a thousand autopsies occurring at the Boston City Hospital during the past three years.

What I wish to show is that strong toxins cause degeneration or necrosis of cells, and exudation, while dilute and weak toxins produce proliferation and phagocytosis. In support of this hypothesis I will cite in part the result of the study of large numbers of cases of certain diseases, in part a limited number of typical and of unusual lesions.

¹ *Journal of Experimental Medicine*, 1898, iii, p. 611.

Certain organisms, such as *Staphylococcus pyogenes aureus* and *Bacillus diphtheriæ*, produce strong toxins which act quickly, and chiefly locally: the effect on the tissues is to produce necrosis and exudation. Other organisms, such as *Bacillus typhosus*, produce mild toxins which act slowly, and cause proliferation or phagocytosis or both.

The stronger toxins, however, can produce these same processes of proliferation and phagocytosis when sufficiently dilute. The best example of this which I have found was in the kidneys of a girl five years old who died in four days with an acute myositis of the right thigh, due to *Staphylococcus pyogenes aureus*. The autopsy was made one hour after death. The femur was not affected. There were multiple miliary abscesses in the lungs, heart, and kidneys. In the kidneys they occurred for the most part in the pyramids, especially towards the bases. Very few of the foci were softened: most of them consisted of areas of necrosis with more or less infiltration with polynuclear leukocytes. Judged by inoculation experiments in rabbits, the lesions were not over 48 hours old.

Many of the lymphatics adjoining these beginning abscesses and running towards the bases of the pyramids were dilated and filled with numerous large phagocytic cells, together with a few lymphoid and plasma cells, and polynuclear leukocytes. The inclusions in the phagocytic cells consisted principally of polynuclear leukocytes and lymphoid cells. Careful study of these lymphatics showed occasional mitosis of the lining endothelium, and much more frequently migration of the endothelial cells through the walls into the adjoining connective tissue. The endothelial cells pass through the wall of a lymphatic in exactly the same way that the polynuclear leukocytes do; namely, a process of the nucleus surrounded by a minimum of protoplasm pierces the wall first, and the rest of the nucleus and the protoplasm gradually follow. After the endothelial cells contained inclusions they still showed the changes of form characteristic of amœboid motion, but none was found in the act of emigration.

In diphtheria the chief lesions are focal and occur for the most part in the air passages, in the immediate vicinity of the bacilli of diph-

theria; but there are other lesions due to the diffusion of a small amount of the toxine through the blood and lymph circulations. The most interesting of the latter are found in the lymph nodules of the tonsils, spleen, and gastro-intestinal tract. They consist of a moderate proliferation of the cells lining the reticulum. This proliferation usually occurs in the centres of the lymph nodules, but sometimes is diffuse or eccentric. Even more marked than the proliferation is the incorporation of the lymphoid cells of the lymph nodules by the cells lining the reticulum and by the new cells formed from them. Phagocytosis like proliferation usually begins in the centres of the lymph nodules and spreads peripherally, but may occur diffusely. In advanced lesions all or nearly all of the lymphoid cells in a lymph nodule may be destroyed. In such cases the centre of a lymph nodule shows a clump of epithelioid cells with the protoplasm more or less fused and containing no inclusions because all of the incorporated cells have been digested, and others are not within reach. Towards the periphery phagocytic cells containing partially digested cells are found, while at the periphery the phagocytic cells are stuffed with lymphoid cells still in a fair state of preservation. The large cells in the centres of the lymph nodules sometimes undergo necrosis, probably in consequence of lack of nutrition.

This brief statement of the proliferation and phagocytosis occurring in diphtheria is based on the histological study of 220 cases of diphtheria, of which a full report will be published shortly by Councilman, Pearce, and myself.

Pratt² has recently shown in a study of 50 cases of acute lobar pneumonia that *Micrococcus lanceolatus* causes marked proliferation of the cells lining the alveoli, the pleural cavities, and the lymphatics: these newly-formed cells are very phagocytic, incorporating and digesting polynuclear leukocytes, lymphoid and plasma cells, and red blood-globules. The proliferation is more marked in the early and in the late stages of the disease than in the middle stage when the exudation of polynuclear leukocytes is abundant.

² Contributions to the Science of Medicine dedicated by his Pupils to William Henry Welch on the Twenty-fifth Anniversary of his Doctorate, p. 265. Baltimore, 1900.

The acute lesions of the glomeruli of the kidney throw much light on the question of proliferation due to bacterial toxins. The organisms most frequently concerned are *Micrococcus lanceolatus* and *Streptococcus pyogenes*. The primary lesion from which the toxin escapes into the circulation is often an acute endocarditis; or the toxin may be secreted directly in the circulating blood in consequence of a septicæmia only. In other cases the primary lesion and the source of the toxin are more remote from the circulation. The lesions produced in the glomeruli vary from necrosis and exudation to pure proliferation.

The toxin secreted by *Micrococcus lanceolatus* causes proliferation almost exclusively of the endothelial cells lining the capillaries of the glomeruli. In one case (No. 1178) of acute endocarditis, in which death occurred at the end of four weeks, the kidneys showed an essentially pure type of acute proliferative intra-capillary glomerulonephritis. Mitotic figures in the endothelial cells were numerous; repeatedly two were found in one section of a single glomerulus.

With *Streptococcus pyogenes* the lesion produced in the glomeruli varies greatly; sometimes it is entirely exudative; at other times there is marked proliferation of the capsular epithelium or of the capillary endothelium: proliferation and exudation are often combined in various proportions.

In one case (B. C. II., No. 98.67) of streptococcus endocarditis and septicæmia there was marked proliferation of the capsular epithelium and also in places of the epithelium at the beginning of the tubules; in one place two mitotic figures in the renal epithelium were found side by side. Many mitoses were found in the capsular epithelium. In some of the capsular spaces there were a little fibrin and a few polynuclear leukocytes. Occasionally phagocytic cells were found within the capsular space: the inclusions consisted of polynuclear leukocytes and red blood-globules.

Certain organisms, such as the typhoid and tubercle bacilli, produce mild toxins which act slowly and usually produce proliferation only, but under certain conditions, especially when the organisms are massed together in large numbers, the toxins may be concentrated and produce necrosis and a purulent exudation.

In typhoid fever a mild diffusible toxine is formed which causes great proliferation of the endothelial cells lining the lymphatics, and the reticulum of the lymphoid tissue of the intestine and the mesenteric lymph nodes; it also causes proliferation of the endothelial cells lining the blood-vessels of the intestine, liver, and spleen. All of these newly formed cells are extremely phagocytic and incorporate lymphoid and plasma cells, polynuclear leukocytes, and red blood-corpuscles. Phagocytic cells formed within the blood-vessels are often carried by the circulation to the liver when they give rise to miliary infarctions by blocking up the capillaries. In the spleen the phagocytic cells may occlude the blood sinuses, giving rise to necroses.

Occasionally, and almost invariably following an attack of typhoid fever, the typhoid bacillus finds suitable conditions for abundant growth. Under these circumstances it produces a marked local reaction, namely, necrosis and purulent exudation like the more virulent organisms already mentioned.

The tubercle bacillus produces a variety of lesions. The typical lesion is of course the miliary tubercle, a clump of epithelioid cells produced by proliferation from endothelial and connective tissue cells. In some situations, as in the lungs, epithelial cells may also take part in the proliferation. Giant cells may or may not be formed. Ordinarily these epithelioid cells show little evidence of phagocytosis, but in certain situations, such as in the meninges of the brain, and in the lymph sinuses of lymph nodes, there may occur a very extensive diffuse proliferation of the endothelial cells which distend the sinuses widely and show marked phagocytic properties; so that an early diffuse tuberculosis of a bronchial lymph node may very closely resemble a mesenteric lymph node of the early, hyperplastic stage of typhoid fever.

On the other hand, in caseous pneumonia, the exudation is often as marked as the proliferation; and in certain rare cases the tubercle bacillus acts like the pus organisms and produces a purulent exudation. In these cases the organisms are present in great numbers, and the toxine is probably much more concentrated than usual.

In the lesions thus far considered, proliferation of endothelial cells

has played a very important part. It can be caused apparently by a great variety of toxins; several examples have been given. This proliferation always takes place by mitosis; careful search of fresh, well preserved tissue has always shown enough typical mitotic figures to account for the newly formed cells. In all of the situations in which the endothelium proliferates, except the glomeruli, the newly formed cells are extremely phagocytic. They incorporate a certain definite group of cells, namely, polynuclear leukocytes, lymphoid and plasma cells, and red blood-globules; they never incorporate epithelial or other endothelial cells. The included cells seem normal at the time they are taken up; occasionally the lymphoid cells have been in mitosis. After inclusion for a time in the phagocytic cells they show more or less evidence of degeneration; the nucleus first stains intensely, then dissolves more or less irregularly, and finally disappears.

The kind of cells which a phagocyte incorporates, depends on its situation in the tissue; if it lies in lymphoid tissue, it takes up lymphoid cells; if in a blood sinus of the spleen, it encloses red blood-globules, often a dozen or more.

In acute proliferative intracapillary glomerulonephritis I have never been able to find any evidence of phagocytosis.

Phagocytic cells necessarily have the power of amœboid motion, otherwise they could not incorporate other cells. In one very perfectly preserved case, the first mentioned in this paper, the young endothelial cells formed by proliferation from the lining endothelium of the lymphatics were found actively emigrating from the vessels into the surrounding tissue.

Under certain circumstances bacterial toxins cause proliferation also of epithelial cells. This is shown most convincingly in cases of acute capsular glomerulonephritis; mitotic figures are easily demonstrable and are often comparatively numerous. Occasionally the more highly differentiated epithelial cells at the beginning of the renal tubules also show active proliferation. Rarely these new-formed cells in the capsular spaces contain inclusions, usually polynuclear leukocytes or red blood-globules. They probably would contain more inclusions if the proper cells were within reach. Proliferation of

epithelium also takes place in pneumonia in the alveoli and in the pleural cavities; mitotic figures as a rule are readily found. The newly formed epithelial cells are desquamated often in large numbers and seem as phagocytic as endothelial cells, which they resemble in every way. The best proof that epithelial cells are phagocytic is the fact that they often can be found containing cell inclusions while they still are attached to the walls of the alveoli.

It is to be noticed that the epithelial cells which have been demonstrated to proliferate and to become phagocytic under the action of bacterial toxins are all cells of a low, undifferentiated type; they are flat cells which resemble endothelial cells and probably perform much the same functions.

There are two situations in which I have found phagocytic cells where their origin is less easily determined.

In one kidney of a very young child (B. C. H., No. 98.233), who died of diphtheria complicated with focal pneumonia and suppuration of both middle ears, there were found several small grayish areas, the largest 4 mm. in diameter, running from the papillæ of the pyramids into the cortex. The areas were rather soft but not fluid; in places small hæmorrhages had taken place into them. Unfortunately no cultures were made from these small areas which microscopically showed an acute pyelonephritis. The tubules in the pyramids were dilated and filled chiefly with phagocytic cells; a few polynuclear leukocytes were present, but most of them were enclosed within the phagocytes. In the connective tissue between the tubules there were many phagocytic cells and some polynuclear leukocytes, lymphoid and plasma cells. In the cortex the areas consisted almost entirely of phagocytic cells lying in the tubules and in the intertubular tissue. In some places all evidence of tubules had disappeared and there existed large areas of phagocytic cells with here and there a few polynuclear leukocytes, and lymphoid and plasma cells between them. In the pyramids many of the phagocytic cells contained large numbers of bacilli which in their morphology and staining reactions resembled in every respect the colon bacillus. The organisms also occurred to some extent outside of the cells; in the cortex they were much fewer in number and occurred mostly in clumps.

So far as could be determined from a study of the sections the phagocytic cells arose entirely in the intertubular tissue. Several of them were found immigrating into the tubules, having passed but part way through the basement membrane. They were also often present in numbers between the renal cells and the basement membrane, apparently having pushed the cells from the wall. In other instances they had distended the lumina of the tubules and flattened the epithelial cells.

The second case is more difficult. In the epididymis of an actively functioning testicle removed from a man 72 years old in consequence of hypertrophied prostate, there were found great numbers of perfectly formed spermatozoa and also numerous huge phagocytic cells incorporating and digesting the spermatozoa by the hundreds. These phagocytic cells cannot be polynuclear leukocytes because the latter are never phagocytic for other cells. They can be only epithelial or endothelial cells; the latter view seems the more probable because it is difficult to conceive of the rather highly differentiated epithelial cells lining the epididymis being transformed into phagocytic cells.

The question whether bacterial toxins can cause connective tissue cells to proliferate and to become phagocytic is not easy to demonstrate. The best evidence is furnished, perhaps, by tuberculosis, but it is not conclusive. It is easy to show that a good deal of connective-tissue reticulum is formed between the epithelioid cells in the periphery of a miliary tubercle and also outside of the tubercle, but that does not prove that the epithelioid cells have produced that reticulum and therefore necessarily are connective-tissue cells. Wherever lymphoid and plasma cells are collected in numbers, a reticulum always forms between them. In the more chronic forms of capsular glomerulonephritis, a connective-tissue reticulum extends in from the capsule and out from the glomerular tuft, and spreads gradually between the epithelial cells in places forming a sort of basement membrane on which they tend to arrange themselves. In this way the capsular space is often divided into a number of small gland-like cavities. In neither of these two cases is it possible for the connective-tissue reticulum to have been produced by the cells which lie in its meshes.

The reticulum seems to grow in between the cells because there is a physiological need of it.

It is not improbable that a good many of the phagocytic cells in the typhoid lesions of the intestine, especially in the muscular coat, are derived from connective-tissue cells; but it is not easy to demonstrate it, partly because endothelial cells occur practically everywhere, partly because the connective-tissue cell, away from the intercellular substance it produces, possesses no very definite characteristics.

In connection with the subject of this paper the following case (B. C. H., No. 98.252) is of interest. A woman 48 years old, with alcoholic history, died of hypertrophic cirrhosis after symptoms lasting about seven months. The inner surface of the bladder except at the neck was found everywhere studded with slightly elevated, flattened, grayish to yellowish-gray nodules of a rather translucent appearance, varying in size from 1 mm. to 2 cm. in diameter. Only occasionally were the edges of the nodules overhanging. They projected from 1 to 4 mm. above the surface, and seemed to lie wholly within the mucous membrane as they were freely movable over the underlying muscle tissue. Two or three of the nodules showed slight hæmorrhages. So far as could be made out the nodules were not subdivided into papillæ, *i. e.* they did not present a cauliflower appearance. The mucous membrane over the nodules was smooth and glistening.

The condition was diagnosticated at first as multiple papillomata of the bladder.

Microscopic examination of the nodules after fixation of the tissue in Zenker's fluid, three hours after death, showed that they were due to collections of large phagocytic cells in the lymph spaces of the upper part of the submucosa. The cells were always most abundant just beneath the epithelium; in the smaller nodules they were confined to this situation, but in the larger nodules they extended more or less deeply into the submucosa, but never so far as to reach the underlying muscle tissue. The capillaries and larger blood-vessels in the nodules were more or less congested, and the tissue around them showed marked infiltration with lymphoid and plasma cells.

There were also numerous lymphoid and plasma cells beneath the mucous membrane and around the vessels between the nodules. The epithelium of the mucous membrane was usually well preserved. Over the nodules it was much thinned, and in places was reduced to a single layer of cells or was even missing.

The cells in the nodules varied a good deal in size, but usually were very large, and round, polygonal, or irregular in shape; the protoplasm as a rule was homogeneous and stained rather deeply with eosin. The nuclei usually single but sometimes two in number, were vesicular in character, round, oval, or irregular in shape, and usually eccentrically situated.

The inclusions in these large cells were of two sorts, bacteria and cells. The bacteria were short rods, in general occurring in small closely aggregated clumps which often lay within vacuoles. The number of organisms in a clump varied from half a dozen to dozens. Some cells contained as many as 7 or 8 clumps of organisms. Other cells contained from a few to hundreds of these organisms scattered diffusely in the protoplasm. The bacteria were found almost entirely within the phagocytic cells; some of them stained sharply, others faintly; many were evidently dead and stained faintly or deeply with eosin. In the cells where the organisms seemed to be growing most rapidly they were usually shorter and plumper than in the cells which contained fewer of them. Occasionally short chains of 2 to 4 members were found. The Gram staining method gave negative results. It is impossible of course to say what the organism is. Morphologically and tinctorially it belongs to the colon-typhoid group.

In places, especially within the larger nodules, nearly every phagocytic cell contained organisms. In other places they were not found in more than one cell in twenty. They were always more numerous near the surface of the nodules than in the deeper portions.

The cells included in the phagocytes were chiefly polynuclear leukocytes and lymphoid cells. Occasionally red blood-globules were incorporated. Most of the inclusions were so far digested that their nature could not be made out. The number of inclusions in a cell varied greatly, but rarely exceeded four or five, although occasionally

there were over a dozen. As a rule the inclusions lay within vacuoles. Within the protoplasm of many of the phagocytic cells were small hyaline bodies of varying size, stained pink and lying in vacuoles. Evidently they were the remains of incorporated cells. Many of them resembled closely the inclusions in the cells of carcinoma.

The nature of these phagocytic cells is difficult to determine absolutely. They resemble in all respects the phagocytic cells found in the lesions of typhoid fever. In all probability they are endothelial cells; but it is difficult to exclude entirely an origin from connective-tissue cells. Where the phagocytic cells were numerous, very little evidence of connective tissue could be found. At the bases of the nodules single phagocytic cells were present in the submucosa lying apparently in spaces between the strands of fibrous tissue. The inclusions in this situation consisted chiefly of hyaline, often concentrically layered, refractive, spherical bodies. The cells often showed forms suggestive of amœboid motion. They seemed to lie in lymph spaces. Distinct lymphatic vessels were very few in number; they contained phagocytic, lymphoid, and plasma cells. In the mucous membrane covering the nodules, phagocytic cells were often found, sometimes in considerable numbers, between the epithelial cells. They probably had migrated to that situation from the underlying tissue. Occasionally in some of the nodules there were small collections of leukocytes, attracted, so far as could be made out, by degeneration of phagocytic cells.

It seems reasonable to conclude that these tumor-like nodules composed of phagocytic cells lying in the submucous tissue of the bladder are due to the action of the organisms found in such large numbers within many of the cells. Certainly the nodules have none of the characteristics of a true tumor formation.

So far I have considered the action of toxins on only three forms of cells. Their action on three other forms I will mention briefly.

The great increase in the number of the polynuclear leukocytes in the blood in infections with certain bacteria, especially the pyogenic organisms, is well known. The leukocytosis produced is of diagnostic

importance; so also is the increase in the number of eosinophiles in trichinosis.³

Of greater significance in this connection is the increase of lymphoid and plasma cells which takes place in certain cases of scarlet fever and diphtheria. This has been considered at length by Councilman in his paper on Acute Interstitial Nephritis.⁴ The cells collect in the veins of the pyramids in the kidney; they emigrate from the vessels into the intertubular tissue and continue to proliferate there; by their numbers they interfere with the function of the kidney and may cause destruction of the tubules. The process in some respects closely resembles a malignant growth, such as leukaemia or lymphosarcoma.

The tendency of the cells which proliferate under the action of toxins is to degenerate and disappear as soon as the agent to which they owe their existence is destroyed or is neutralized. The best example is afforded, perhaps, by the resolution which takes place in the hyperplastic patches of Peyer and in the mesenteric lymph nodes in typhoid fever. The affected tissues quickly recover their normal appearance. In certain situations the results of the proliferation are more permanent and more disastrous. For example, the renal glomeruli may be destroyed partially or entirely by occlusion of the capillaries, or by the formation of fibrous tissue in the capsular spaces, and be converted into small contracted masses of hyaline connective tissue.

The processes of proliferation and phagocytosis which I have been describing as due directly to the action of toxins are regarded generally as reparative in nature. Similar processes certainly occur in repair. Is it possible to differentiate them?

In repair, cells proliferate for definite purposes—epithelium to cover denuded surfaces; connective tissue and lymph endothelial cells to replace losses of tissue and to remove foreign bodies, such as necrotic cells, fibrin, fat, myelin, etc.; blood endothelium to form new blood-vessels for the nourishment of the new tissue.

The cells, however, which proliferate under the direct action of

³ Brown, Trichinosis, with especial reference to the increase of the eosinophilic cells in the blood and muscle. *Journal of Experimental Medicine*, 1898, iii, p. 315.

⁴ *Journal of Experimental Medicine*, 1898, iii, p. 393.

toxines, multiply greatly in excess of need, and show a lack of definite purpose; they may exert some more or less beneficent action, such as possibly the production of antitoxines, but they can no more be regarded as reparative than the exudation called out by the toxines; both may work infinite harm. As we have seen in the cases cited, phagocytic cells may block up lymphatics and undergo necrosis; they may be carried to the liver as emboli, blocking up the capillaries and giving rise to focal necroses of liver cells; they may enclose and destroy the lymphoid cells in lymph nodules and then undergo necrosis themselves; they may occlude the veins in the spleen, giving rise to infarctions; they may occlude the capillaries of the glomeruli by growth within the vessels or by pressure from without; they may give rise to tumor-like formations. Lymphoid and plasma cells may multiply in the circulation, and may invade and multiply in the kidney interfering with its functions and causing its destruction. The phagocytic cells are phagocytic beyond all bounds of necessity and destroy great numbers of active, useful cells. These are all abnormal and to a certain degree malignant properties.

A CONTRIBUTION TO STAINING METHODS.

- I. A DIFFERENTIAL STAIN FOR CONNECTIVE-TISSUE FIBRILLÆ AND RETICULUM.
- II. CHLORIDE OF IRON HÆMATOXYLIN FOR NUCLEI AND FIBRIN.
- III. PHOSPHOTUNGSTIC ACID HÆMATOXYLIN FOR NEUROGLIA FIBRES.

By F. B. MALLORY, M. D.

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I. A DIFFERENTIAL STAIN FOR CONNECTIVE-TISSUE FIBRILLÆ AND RETICULUM.

The following method for staining connective tissue is simple and is believed to be better than any yet proposed for that purpose. It is not absolutely differential because, besides connective-tissue fibrillæ and reticulum, it also stains certain hyaline substances, but these latter usually are so different morphologically that confusion cannot arise. The method is also useful for the study of fibrin, smooth and striated muscle fibres, and amyloid. The manner of staining is in brief as follows:

1. Fix in corrosive sublimate solution or in Zenker's fluid.
2. Embed in celloidin or paraffin.
3. Stain sections in a $\frac{1}{20}$ to $\frac{1}{10}$ of a one per cent aqueous solution of acid fuchsin 1 to 3 minutes.
4. Wash in water.
5. Place in a 1 per cent aqueous solution of phosphomolybdic acid for 1 minute or longer (use platinum or glass needle).
6. Wash in two changes of water.
7. Stain in the following solution for 2 to 20 minutes or longer:

Aniline blue soluble in water (Grübler),	0.5
Orange G (Grübler)	2.0
Oxalic acid	2.0
Water	100.0
8. Wash in water.
9. Dehydrate in 95 per cent alcohol.

10. Blot on the slide and clear in xylol, or clear in oleum origanici.

11. Xylol balsam.

The fibrillæ and reticulum of connective tissue, amyloid, mucus, and certain other hyaline substances stain blue; nuclei, protoplasm, elastic fibres, axis cylinders, neuroglia fibres, and fibrin, red; red-blood globules and myelin sheaths, yellow. The various structures do not stain with equal intensity, so that certain ones are brought out with great sharpness. This is particularly true of the fibrillæ and reticulum of connective tissue, also of fibrin and smooth and striated muscle fibres.

If it is desired to bring out the connective tissue as sharply as possible, omit the staining with acid fuchsin. Then the nuclei and protoplasm stain yellow and the blue fibrillæ and reticulum stand out more prominently.

The method is satisfactory only after hardening in corrosive sublimate solution or in Zenker's fluid, although fair results can be obtained after fixation by the method I have recommended for neuroglia fibres,¹ but the latter procedure is best suited to the central nervous system, under which subject it will be mentioned later. With alcohol and other fixatives everything stains blue.

The oxalic acid makes the aniline blue stain more intensely and quickly than it otherwise would. The orange G tends to limit the blue to connective tissue. The phosphomolybdic acid has two functions: it intensifies and fixes the acid fuchsin in certain histological elements while removing it from the connective tissue; it slows the action of the aniline blue and prevents it from gradually staining everything else in addition to the connective tissue.

The time of staining varies somewhat according to the structures it is desired to render prominent, the character of the tissue, and the thickness of the sections. Paraffin sections will stand longer staining than thick celloidin sections.

Elastic fibres stain pale pink or yellow. Sometimes the elastic laminæ of arteries seem to stain blue, but this is because the connec-

¹ *Journal of Experimental Medicine*, 1897, ii, p. 532.

tive tissue closely applied to the plates of elastic tissue stains deeply and conceals them.

This method is also useful in the study of fibrin, and of smooth and striated muscle fibres because they are brought out in sharp contrast to the connective tissue, especially if the staining with acid fuchsin is prolonged a little or if the sections are washed out for a few minutes in alcohol, which removes aniline blue rather quickly but does not affect the acid fuchsin.

The stain brings out clearly the deposit of amyloid in the tissues, especially the liver, because amyloid stains a light blue and stands out with the greatest sharpness from the red protoplasm of the liver cells. In places, where they have not degenerated, the connective-tissue fibrillæ can be seen running through or along side of the amyloid material.

The mucus in epithelial cells stains blue, as do also renal casts and certain other hyaline substances.

The method can be used also for the study of the central nervous system. It may be used after fixation in corrosive sublimate solution or in Zenker's fluid as already directed, or better still with slight modifications after the method I have recommended for the fixation of neuroglia fibres, namely:

Fixation.

1. Fixation of thin pieces of nervous tissue (2 to 5 mm. thick) in a 4 per cent aqueous solution of formaldehyde (10 per cent formol) for at least 4 days.
2. Saturated aqueous solution of picric acid 4 days or more.
3. 5 per cent aqueous solution of bichromate of ammonium 4 days in the incubator (use an abundance of the solution and change at the end of 24 hours so as to avoid any chance of a precipitate).
4. Alcohol. Embed in celloidin.

Staining Method.

1. 1 per cent aqueous solution of acid fuchsin, 2 to 5 minutes.
2. Wash quickly in water.
3. 1 per cent aqueous solution of phosphomolybdic acid, 1 to 2 minutes.
4. Wash in two changes of water.

5. 1 to 3 minutes in the aniline blue and orange G solution.
6. Wash in water.
7. Dehydrate in 95 per cent alcohol.
8. Blot and clear with xylol, or use *oleum origani cretici*.
9. Xylol balsam.

Connective tissue blue; neuroglia fibres deep red; axis cylinders and ganglion cells a lighter red.

II. CHLORIDE OF IRON HÆMATOXYLIN FOR NUCLEI AND FIBRIN.

The results which may be obtained by this method are equally quick and satisfactory after all of the usual fixing reagents, except, perhaps, formaldehyde.

Celloidin or paraffin can be employed for embedding.

1. Stain sections on the slide for 3 to 5 minutes in a 10 per cent aqueous solution of ferric chloride.
2. Drain and blot the sections; then pour over them a few drops of a freshly prepared 1 per cent aqueous solution of hæmatoxylin. If all of the hæmatoxylin is precipitated by the excess of the ferric chloride, pour off the solution and add a fresh supply. In 3 to 5 minutes the sections will be colored a dark bluish black.
3. Wash in water.
4. Decolorize and differentiate in a $\frac{1}{4}$ per cent aqueous solution of ferric chloride. The sections should be kept constantly moving in the solution. The differentiation will be complete in a few seconds to one or more minutes.
5. Wash in water.
6. Dehydrate in alcohol.
7. Clear in *oleum origani cretici*.
8. Xylol balsam.

In the above directions definite strengths have been assigned to the solutions, but they may vary greatly without affecting the result. The important point is to get the sections stained deeply, and then to decolorize slowly. The differentiation can be stopped at any moment by transferring the sections to water. Sometimes it is advisable to examine the sections under the microscope to see if enough color has been extracted.

The strength of the hæmatoxylin solution is unimportant; it is

simply necessary to have enough hæmatoxylin to combine with all of the iron in and on the section. The simplest way is to dissolve by the aid of heat a pinch of the crystals in a few cubic centimetres of water in a test-tube. A little experience will determine about how much is needed. If a solution of hæmatoxylin more than one or two days old is used, the color obtained is grayish-blue and not so bright.

This method gives a sharp, permanent, dark blue stain to nuclei; it also stains fibrin of a grayish to dark blue color; if the decolorization is not carried too far, the contractile elements of striated muscle are brought out very sharply. In Zenker preparations the red globules appear of a greenish-gray color. Connective tissue is tinted a pale yellow. The nucleus of *Amœba coli* stains sharply by this method.

III. PHOSPHOTUNGSTIC ACID HÆMATOXYLIN FOR NEUROGLIA FIBRES.

This method was published originally in the *JOURNAL OF EXPERIMENTAL MEDICINE* (1898, III, p. 611). At that time the phosphotungstic acid manufactured by Merck was not pure. It contained a trace of phosphomolybdic acid and also some oxidizing agent which ripened hæmatoxylin at once. The staining solution made up according to the directions then given but with the pure phosphotungstic acid now manufactured gives negative results because the hæmatoxylin will not ripen spontaneously even after standing for months. The solution can be ripened, however, at once by the methods advised for alum hæmatoxylin; the best results are obtained by using peroxide of hydrogen.

To render the stain somewhat sharper, and to prevent the myelin sheaths from taking a bluish tint when the time of staining is prolonged, it has been found advisable to treat the sections with permanganate of potassium followed by oxalic acid.

The tissue should be fixed by the formaldehyde, picric acid, and bichromate of ammonium method described under the connective-tissue stain.

Staining Method.

1. Place the sections in a $\frac{1}{2}$ per cent aqueous solution of permanganate of potassium for 15 to 30 minutes.

2. Wash in water.
3. 1 per cent aqueous solution of oxalic acid 15 to 30 minutes.
4. Wash in 2 or 3 changes of water.
5. Stain in the following solution 12 to 24 hours or longer.

Hæmatoxylin	0.1
Water	80.0
10 per cent aqueous solution of phosphotungstic acid (Merck)	20.0
Peroxide of hydrogen (U. S. P.).....	0.2

Dissolve the hæmatoxylin in a little water by the aid of heat and add it, after cooling, to the rest of the water and the acid. Then add the peroxide of hydrogen. The solution keeps perfectly and can be used repeatedly.

6. Wash quickly in water.
7. Dehydrate quickly in 95 per cent alcohol.
8. Oleum origani cretici.
9. Xylol balsam.

Nuclei, neuroglia fibres, and fibrin, stain blue; axis cylinders and ganglion cells, pale pink; connective tissue, deep pink.

The blue color is a little sensitive to strong light, and on prolonged exposure will fade to pink.

If a permanent, isolated stain of the neuroglia fibres is desired, place the sections, after staining as directed in the phosphotungstic acid hæmatoxylin and washing in water, in a 30 per cent alcoholic solution of ferric chloride for 5 to 20 minutes (rarely longer); then wash in water and dehydrate as before. The nuclei, neuroglia fibres, and fibrin stand out sharply of a clear blue color; everything else is decolorized or appears of a pale yellowish or gray tint. The results obtained by this last step are practically identical with those obtained by either of the modified fibrin stains, and the method has the decided advantage of being applicable to any number of sections at once. The method will be found to bring out rather sharply in the nerve fibres outside of the cord the funnel-shaped markings in the myelin sheaths.

Phosphotungstic acid hæmatoxylin will be found occasionally useful as a stain for ordinary tissues hardened in various fixatives. Two to twenty-hours usually are required for staining. Nuclei, fibrin, elastic fibres, and the contractile elements of striated muscle stain blue; the other tissue elements stain pink to red.

A STUDY OF THE NEUROFIBRILS¹ IN THE GANGLION CELLS OF THE CEREBRAL CORTEX.

BY STEWART PATON, JOHNS HOPKINS UNIVERSITY.

PLATE I.

As has often been pointed out, the use of the terms chromatic and achromatic to distinguish certain characteristics of the ganglion cell is misleading. The words are only applicable to describe the stained and unstained tracts in nerve cells which have been stained by the Nissl methylene-blue method. In order to avoid confusion in describing the morphological characteristics of the ganglion cells, it has seemed to the writer advisable to use terms which as far as possible admit of general application and do not have reference simply to the results given by one particular stain. For this reason Marinesco's classification of the constituents of the ganglion cell into (1) chromatic, (2) achromatic and (3) amorphous elements, is open to criticism. In the present state of our knowledge of the morphology of the ganglion cell it is not possible to give more than a tentative classification of the cellular constituents. The various elements of the nerve cell may be roughly grouped as follows: (1) cytoplasm, (2) Nissl bodies, (3) fibrils, (4) constituents whose morphological characteristics are not yet definitely known.

The achromatic tracts are made up of at least two constituents, the cytoplasm and a definite specific fibrillary substance which possesses morphological characteristics by which it can be easily differentiated from the surrounding protoplasm in which it is held. There is also another sense in which the use of the term achromatic is not exact. Under certain conditions the achromatic tracts exhibit marked chromophilic tendencies. This is often due to the fact that the fibrils

¹ In this paper the term neurofibril is used in a purely morphological sense to describe the fibrils which lie wholly or only partly within the ganglion cells of the cerebral cortex.

are capable of being stained by reagents for which under normal conditions they have little or no affinity. In view of the fact that the fibrils are now known to be an important and definite constituent of the ganglion cell and on account of the present confusion of terms in cytological nomenclature it has seemed best to make the foregoing suggestions in reference to the selection of terms.

Although it is often possible to stain the fibrils by various methods there are none that are satisfactory except the hæmatin or the gold stains of Apáthy and the as yet unpublished methods of Bethe. As Apáthy's methods are somewhat capricious when an attempt is made to stain the fibrils in the ganglion cells in the higher vertebrates, I have decided to publish a modified hæmatin method which often, but not always, gives excellent results. I hope others may be induced to try the method and be able to propose further modification in the technique which will make the method capable of more general application.

The sections represented in Plate I, Figs. 1 and 2, were both taken from the cerebral cortex of a fully developed pig. The tissue immediately after death was placed for 24 hours in a saturated solution of bichloride of mercury, to which 5% of glacial acetic acid had been previously added. Other methods of fixation can also be used. After the fixation the tissue is transferred to 95% alcohol. The alcohol must be changed twice during the first 24 hours and then every other day for a week. After this the fluid need not be changed oftener than once a week. In two weeks the sublimate is thoroughly removed from the tissues. I believe that better and more reliable results are obtained by not using the iodine solutions to remove the sublimate. This chemical has a very deleterious effect on the tissues. After being hardened in alcohol the tissue is ready for embedding. Either celloidin or paraffin may be used, preferably the latter, care being taken to use chloroform instead of xylol. The sections are fixed to the slide by the use of distilled water, and, after drying and the removal of the paraffin by chloroform, are put into 95% alcohol. The slide is then put for 1-2 hours into tinctura ferri Rademacheri as recommended by Weigert in his "mitosis stain." The sections are quickly rinsed in distilled water and are stained for 24 hours in the Apáthy² hæmatin solution. The

² *Mittheil. aus der zoolog. Station zu Neapel*, 1897, xii, Hft. 4.

whole section is then found to be over-stained. The preparations are then bleached under the microscope in an aniline oil and alcohol mixture (one part of the former to nine parts of 70% alcohol). The slides are finally well washed in tap-water, rinsed in distilled water, and after dehydrating and clearing are mounted in the usual way in Canada balsam dissolved in chloroform. The specimens when once fixed can be kept indefinitely.

As said already, the method is not yet entirely satisfactory. In the human nervous system the results are only very rarely good; but I believe this is due to the fact that it is so difficult to obtain perfectly fresh material. The ganglion cells in the human cerebral cortex when stained by this method, as a rule, give pictures exactly similar to those obtained when sections have been made from the brains of animals which have not been fixed immediately after death. In both cases the fibrils are only very imperfectly stained. This fact has an important bearing if there are those who still contend that the fibrils are artefacts, or are only the results of post-mortem changes. Arnold³ has said recently that he was unable to find any long fibrils in the ganglion cell, but only short ones together with small granular masses. His observations were made on the ganglion cells in the human nervous system. The continuity of the fibrils after death is soon broken and there follows a period of granular degeneration. As a rule, I believe, the fibrils are very susceptible to post-mortem changes, and these changes are first seen in the body of the cell. Apparently the fibrils in the processes, particularly in the apical process of the pyramidal cells, remain for a considerable period after death unchanged. This may account for v. Lenhossék's statement in regard to the fibrillary substance. He admits the presence of the fibrils in the apical processes, but does not believe that the fibrils exist in the cell body. It can be very easily shown that the post-mortem degeneration of the fibrils begins in the cell body, and in specimens which are imperfectly fixed the cell body often has a granular appearance in strong contrast to the apical processes where the fibrils can be plainly seen.

In both sections from which the drawings for Plate I were made, it is possible to follow fibrils which run through nearly the whole

³ *Archiv f. mikr. Anat. u. Entwickl.*, 1898, lii, p. 535.

length of the cell. Similar fibrils have been described by Bethe. In sections thicker than those represented in the plate, fibrils can often be seen running through the whole length of the cell. As already stated, Arnold does not believe that any long fibrils are found in the ganglion cell, but only "short fibrils and granular masses." I am convinced that the failure of Arnold to find longer fibrils in the ganglion cell is due to the technique which he has employed. The use of strong iodine solutions makes the staining of the fibrils difficult and often impossible.

In comparing Figs. 1 and 2 certain important differences are recognizable. In the section represented in Fig. 1 the fibrils run straight through the cell processes without being connected with each other, but in the cell body there are connections between the individual fibres, so that a very wide-meshed network is formed. This agrees with the description given by Apáthy, and does not support Bethe's view. The latter failed to find any network formed within the cell body. In the apical process of the ganglion cell, shown in Fig. 2, it is easy to distinguish the fibrils, many of which can be followed through the whole length of the process and well into the cell body. In the preparation from which the drawing was made a bundle of fibrils can be followed from a point at about the outer fourth of the apical process through the remainder of the process into the cell body almost to its base, near where the axone is given off. Another bundle of fibrils can be followed which enter the ganglion cell from the ground substance on one side at about the level of the nucleus. This bundle passes inward and downward below the nucleus, and leaves the cell by one of the basal processes on the opposite side of the cell.

In the section from which the drawing for Fig. 2 was made it can easily be seen that the diffuse network of fibrils which is visible is not within, but is superimposed on, the cell and its processes. The fibrils here lie in a different and more superficial plane than that of the cell and its processes. I am convinced from my own observations that it is this extracellular network of fibrils which Held⁴ has described as a pericellular network of terminal axones. The same is

⁴*Arch. f. Anat. u. Entwicklungsgesch.*, 1897, Suppl.-Bd., p. 273.

true also of the reticulum surrounding the nerve cell described by Golgi.⁵ It is altogether impossible by means of the silver stains to differentiate between the fibrils and the processes of the cells, so that these methods are no longer of any service in studying the structure of the ganglion cells. By this modified hæmatin method it is possible to demonstrate the passage of the fibrils from the cell body, as well as from the processes, into the intercellular substance. The discussion of the relation of the neurofibrils to the intranuclear network as well as to the grey substance will not be considered in this paper.

After a careful study of the development of the ganglion cell⁶ I have been brought to the conclusion that the presence or absence of "gemmules" is dependent upon the existence or non-existence of the fibrils. Hill⁷ concluded that these lateral buds or gemmules were nothing more or less than "the cell end of an unstainable nerve filament." This is, I believe, only partly true. A gemmule is formed by a silver deposit at the point where the nerve filament or fibril enters the cell process, and it is also found if the fibril is simply lying upon the process as is the case with most of the fibrils in Fig. 2. The gemmules are found either at the point of entrance, or at the point of simple contact of a fibril with the protoplasm of the cell process. It can be easily shown that the appearance of the gemmules in the cortical cells of embryos is synchronous with the appearance of the fibrils. In the adult as well, the gemmules depend upon the existence of the fibrils and any pathological process which destroys the latter causes a corresponding diminution or absence of the former.

DESCRIPTION OF PLATE I.

Both sections are from the cerebral cortex of a fully developed pig. Thickness of sections 2μ . Zeiss, Apochromat. No. 6, Ocular, 2 mm.

FIG. 1. The neurofibrils can be seen running through the cell-body and apical process. In the section fibrils can be seen entering the ground-substance from the cell-body as well as from the processes.

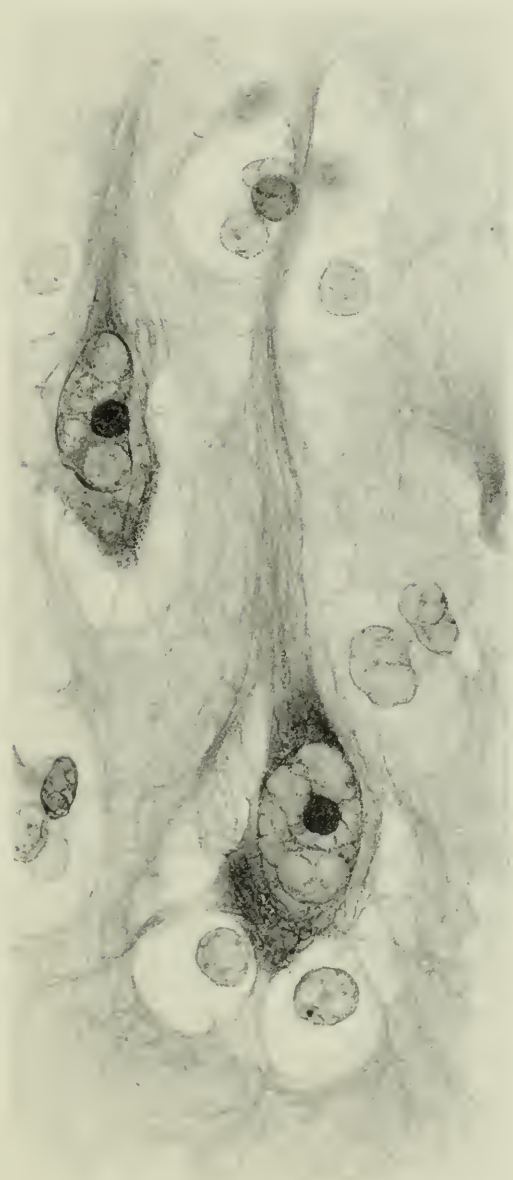
FIG. 2. Most of the fibrils, which look as if they were within the cell-body, are really extracellular, and form part of the fibre-network of the ground-substances.

⁵ *Arch. ital. de biol.*, 1898, xxx, p. 71.

⁶ The histogenesis of the cellular elements of the cerebral cortex. Contributions to the Science of Medicine dedicated by his Pupils to William Henry Welch on the Twenty-fifth Anniversary of his Doctorate, p. 709. Baltimore, 1900.

⁷ *Brain*, London, 1897, xx, p. 131.

26'



Max Brodel, fec.

FIG. 1.



Max Brodel, fec.

FIG. 2.

AN EXPERIMENTAL STUDY OF OXALURIA, WITH SPECIAL REFERENCE TO ITS FERMENTATIVE ORIGIN.

BY HELEN BALDWIN, M. D.

(From the Laboratory of Dr. C. A. Herter, New York City.)

After the discovery of calcium oxalate crystals in the urine by Donné¹ in 1838, there followed a careful study of the symptoms associated with their deposit. This resulted in the description of a so-called oxalic-acid diathesis, which was thought by certain clinicians, notably Prout,² Golding Bird,³ and Begbie,⁴ to be of considerable importance and interest. Smoler,⁵ however, found that the crystals were present in fifty-two per cent of four hundred specimens of urine, and Bacon, in forty-one per cent of nine hundred and nine cases, from patients suffering from almost every form of disease, and Neubauer⁶ showed that calcium oxalate was almost constantly present in solution in the urine, even when not precipitated. These discoveries led to the belief that oxaluria is a symptom of many diseases, and not in itself of grave import. In 1896, Dunlop⁷ published an article which supported the view that all the oxalic acid excreted in the urine had been ingested with the food, and that it was never formed in the animal organism by metabolism.

I have made the following series of observations and experiments: (1) to test the accuracy of Dunlop's conclusion; that is, to determine whether oxalic acid is ever formed in the animal body; (2) to study the influence of the ingestion of oxalic acid in foods upon the amount

¹ Donné, *Compt. rend. Acad. d. Sc.*, Paris, 1839.

² Prout, *On the Nature and Treatment of Stomach and Urinary Diseases*. London, 1840.

³ Golding Bird, *Observations on Urinary Concretions and Deposits*. London, 1842.

⁴ Begbie, *Monthly Journ. Med. Sc.*, Edinburgh and London, 1848-9, ix, p. 943.

⁵ Smoler, *Prager Vrtjschr. f. d. prakt. Heilk.*, 1861, lxix, p. 157; lxx, p. 35.

⁶ Neubauer, *Arch. d. Vereins f. gemeinsch. Arb. z. Förd. d. wiss. Heilk.*, 1860, iv, p. 1. Neubauer und Vogel, *Analyse d. Harns*. Wiesbaden, 1863.

⁷ Dunlop, *Journ. Pathol. and Bacteriol.*, 1896, iii, p. 389.

excreted in the urine; (3) to study the physiological action of soluble oxalates with a view to deciding in what measure the presence of oxalic acid in the system is responsible for the symptoms attributed to the "oxalic-acid diathesis."

Occurrence of Oxalic Acid in Foods.—Oxalic acid and its salts are found widely distributed in nature. They are present in many of the common forms of vegetables, grains and fruits. Esbach⁸ and Auerbach⁹ have made estimations of the oxalic acid in the common foods. Some of those which they found to be rich in oxalic acid are spinach, rhubarb, dried figs, cocoa, tea, coffee, pepper, potatoes, beetroot, green beans, plums, tomatoes and strawberries. Foods which they found to contain little or no oxalic acid are peas, asparagus, cucumbers, mushrooms, onions, lettuce, rice, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

In connection with the following experiments, a few foods were examined in order to select a diet free from oxalates, which should be more liberal than Dunlop's exclusively milk diet. The list of foods free from oxalic acid includes proteids (meat, milk and eggs) with sugar, butter, corn meal, rice, and the Huntley and Palmer breakfast biscuits.¹⁰

Characteristics of Calcium Oxalate Crystals.—When calcium oxalate is rapidly precipitated from a solution it falls in a very finely divided crystalline deposit, appearing like an amorphous sediment. These crystals contain only one molecule of water of crystallization. If allowed to stand a number of days, these crystals may group themselves in dendritic or star forms, similar to certain of the phosphatic crystals. After standing from ten days to three weeks, the characteristic octahedral crystals frequently form. These contain three molecules of water of crystallization.

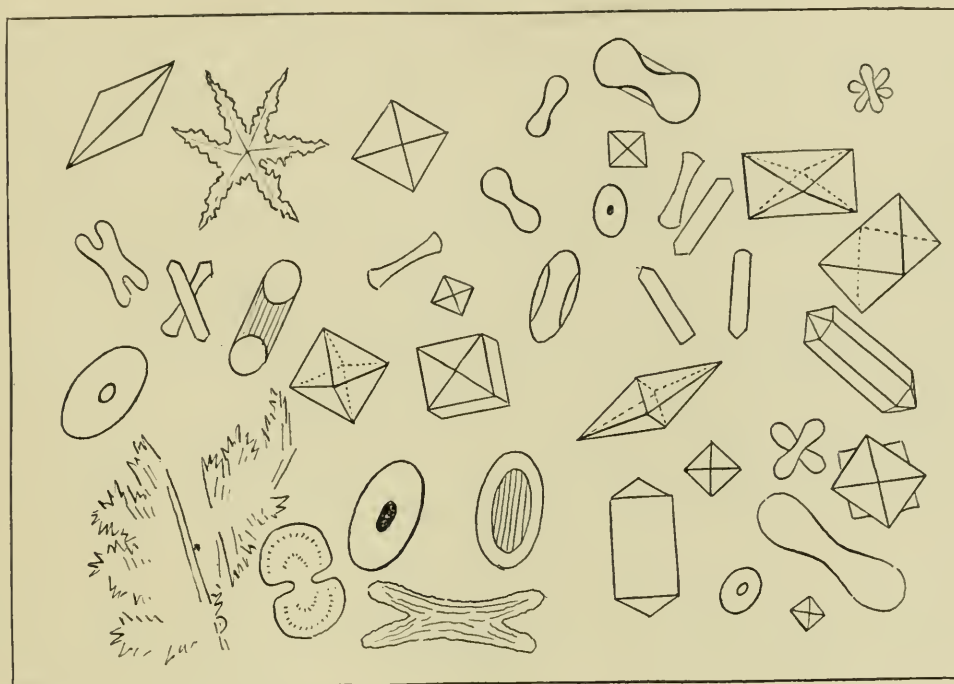
In the urine, calcium oxalate usually forms in octahedra, but it may

⁸ Esbach, *Bull. gén. de thérap.*, Paris, 1883, civ, p. 385.

⁹ Auerbach, *Virchow's Archiv*, 1879, lxxvii, p. 226.

¹⁰ The food tested was cut or ground in small pieces, boiled in dilute hydrochloric acid, allowed to stand forty-eight hours, filtered and washed free from acid. The filtrate was then neutralized with ammonia, rendered very slightly acid with acetic acid, and then treated like the urine by a method described below.

be found in a variety of crystalline forms, described by Fürbringer¹¹ as following two types, the prismatic and spheroidal (see the accompanying figure). These crystals which are clear and colorless are insoluble in ammonia and alcohol, almost insoluble in hot and cold water (1:500,000 (Storer) = 2 mg. per litre), slightly soluble in acetic acid (3-9 mg. in 20-60 cc. dilute acetic acid (Nickel¹²), but are readily dissolved by the strong mineral acids.¹³



Varieties of Calcium Oxalate Crystals.

Quantitative Estimation of the Calcium Oxalate in the Urine.—The quantitative estimation of the calcium oxalate found in the urine is a long and tedious process, and, unless great care is used, it is liable to large percentages of error. The method employed in these experi-

¹¹ Fürbringer, *Deutsch. Arch. f. klin. Med.*, 1875, xvi, p. 519.

¹² Nickel, *Ztschr. f. physiol. Chemie*, 1887, xi, p. 186.

¹³ The solubility of calcium oxalate as tested in this series of experiments was found to be as follows:

1. In cold distilled water 2.2 mg. per litre.
2. In boiling water 1 mg. per litre.
3. In 2.5% acetic acid, after standing 48 hrs., 28 mg. per litre.
4. By washing with 2.5% acetic acid, 8.2 mg. per litre.

ments is founded on that of Dunlop.¹⁴ It differs essentially from the older methods of Neubauer¹⁵ and of Shultzen¹⁶ in that the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride or calcium hydrate.

Dunlop's method (slightly modified). The urine should be thymolized as soon as passed to prevent fermentation and the precipitation of phosphates. If the specimen is alkaline, render it slightly acid with acetic acid.

To 500 cc. of a well mixed specimen of the twenty-four hours' urine, add 150 cc. of over 90% alcohol, to precipitate the calcium oxalate. Set aside for forty-eight hours. Filter, washing the beaker carefully, and removing crystals from the sides by rubbing with a rod protected by rubber tubing. Wash the sediment thoroughly with hot and cold water and with dilute acetic acid (1%). Place the filter in a small beaker and soak in a small amount of dilute hydrochloric acid. Then wash with hot water till there is no further acid reaction; filter the washings and evaporate the filtrate to about 20 cc. Add a very little calcium chloride solution to ensure an excess of calcium; neutralize the hydrochloric acid with ammonia, and then render the solution slightly acid with acetic acid. Add strong alcohol to the amount of 50% of the volume of the fluid and set aside forty-eight hours. Collect the sediment on an ash-free filter, wash with cold water and dilute acetic acid till free from chlorides. (Avoid the use of hot water, as it carries the finely divided precipitate through the pores of the filter). Incinerate first over a Bunsen burner and afterwards for five minutes in a blowpipe flame, cool over sulphuric acid, and weigh. The ash is calcium oxide, each gramme of which represents 1.6 grms. oxalic acid.

In the older methods of Neubauer and of Shultzen, the urine was rendered alkaline, and the oxalic acid precipitated by adding an excess of calcium chloride solution. In this way the phosphates, as well as calcium sulphate, are precipitated, and can with difficulty be separated from calcium oxalate; for, unless large quantities of water and acetic acid are used in washing, traces of phosphates and sulphates remain and are weighed in the ash, whereas, if enough of these is used to dissolve away the impurities, there is a weighable amount of calcium oxalate dissolved and lost. In using the method either of Neubauer, Shultzen, or Dunlop, the ash should each time be tested for phosphates and sulphates.

The following experiments (Table I) were made to test the accuracy of Dunlop's method; 500 cc. of a well mixed twenty-four hours' specimen of urine was examined for the amount of oxalic acid present and the result noted. Then to another 500 cc. of the same specimen was added a weighed amount of calcium oxalate and the mixture treated as in the first instance. When using the utmost care, the loss will be about one milligramme.

¹⁴ Dunlop, *op. cit.*

¹⁵ Neubauer and Vogel (Huppert), *Analyse d. Harns.* Wiesbaden, 1898. (Fürbringer and Czapek's modification of Neubauer's method.)

¹⁶ Shultzen, *Arch. f. Anat. u. Physiol.*, 1868, vi, p. 719.

TABLE I.
EXPERIMENTS TO TEST THE ACCURACY OF DUNLOP'S METHOD.

	Calcium oxalate added to urine.	Calcium oxalate recovered.	Calcium oxalate lost.
1.....	.0289 gm.	.0270 gm.	.0019 gm.
2.....	.0169	.0158	.0011
3.....	.0232	.0256	Trace of phosphates in ash.
4.....	.0203	.0201	.0002 gm.
5.....	.0150	.0150
6.....	.0281	.0282
7.....	.0202	.0208	Faint trace of phosphates.
8.....	.0281	.0280	.0001 gm.
9.....	.0453	.0455	Faint trace of phosphates.
10.....	.0289	.0270	.0019 gm.

Salkowski¹⁷ has described a method of separating the phosphates from the oxalates by shaking out with ether. This does not seem necessary in the case of human urine when Dunlop's method is used. But in dog's urine, with high specific gravity, it is very difficult to remove the phosphates even while using Dunlop's method.

Oxalic Acid in Normal Urine.—In health the amount of oxalic acid excreted in twenty-four hours varies with the amount ingested in the food. The average is estimated by Fühbringer as .02 gm. The following tables show varying amounts excreted with different diets:

TABLE II.
NORMAL URINE. DIET CHIEFLY CARBOHYDRATES. (MEAT, MILK, BREAD, POTATO, OATMEAL.)

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1305	1017	.0058	None.
1235	1020	.0004	Few.
1615	1015	.017	Numerous.
1295	1012	.013	None.

TABLE III.
NORMAL URINE. FOOD RICH IN OXALATES. MIXED DIET WITH LARGE AMOUNTS OF TOMATO, SPINACH, TEA AND COFFEE.

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1765	1011	.0011	Present.
1865	1017	.0012	"
1945	1015	.0006	"
1760	1016	.0028	"

¹⁷ Salkowski, *Centralbl. f. d. med. Wiss.*, 1899, xxxvii, p. 257.

TABLE IV.

			NORMAL URINE.		MIXED DIET.		
Diet.	Vol.	Sp. gr.	Oxalic acid in 24 hrs.	Oxalic acid per litre.	Calcium oxalate crystals in sediment.	Impurities in ash.	
Ordinary mixed diet }	1645	1016	.0047	.0029	None.		
“	1360	1018	.0022	.0016	“		
“	1185	1014	.0099	.0083	“		
“	1065	1019	.002	.0019	“		
“	750	1028	.0017	.0014	Numerous.		
“	865	1026	.0147	.0170	None.	{	Trace of phosphates.
“	730015	.021	Few.		“
“ + rhubarb }	480	1034	.034	.072	{ Large, numerous.	{	Trace of phosphates and sulphates.
Ordinary mixed diet }	800	1022	.024	.030	Numerous.		“
“	1720	1010	.011	.006	Small.		
“	720	1022	.009	.0128	None.		
“	1115	1019	.017	.015	Numerous.	{	Trace of phosphates.
“	1210	1014	.0035	.0014	None.		
“	1205	1013	.0017	.0016	Few.		
“	950	1019	.0076	.008	“		
“	820	1022	.0103	.0116	Very few.		
“	950	1021	.0016	.0006	“ “		
“	950	1024	.0115	.012			
“	1225	1014	.0047	.0038			
“	1250	1013	.0169	.0135			
“	1225	1013	.0031	.0026			
“	1300	1013	.0062	.0048			
“	2000	1012	.0062	.0031			
“	1700	1012	.0011	.0006			

There were also examined thirty-five specimens from patients who were receiving a mixed diet. These patients were suffering from gastric and intestinal disorders. The urinary examinations gave the same results as in the case of the healthy persons. The daily excretion of oxalic acid varied from a few milligrammes to about two centigrammes, the mean falling below ten milligrammes. It was also noted, as Fühbringer had shown, that the precipitation of calcium oxalate crystals seemed to bear no relation to the amount in solution in the urine.

Characteristics of Urines Precipitating Oxalate of Lime.—The conditions causing precipitation of calcium oxalate in the urine are

not known. The characteristics of the urines of oxaluria have been studied by Begbie, Golding Bird, MacLagan and others. Fürbringer has shown that the crystals may be absent when a specimen contains a large amount of oxalic acid or present when there is only a trace. They are found with every degree of acidity, from highly acid to alkaline, with every degree of specific gravity and with every color. The following observations are collected from Dr. Herter's records.

In 370 cases in which the sediment was examined calcium oxalate crystals were found in 94, that is 25.4 per cent. The acidity varied from .091 gm. oxalic acid to alkaline, the mean acidity was .012 gm. The color varied from 1 to 7—mean 4—(Vogel's Chart). Thirty per cent of the specimens contained uric acid or urates in the sediment. The ratio of urea to uric acid varied from 23.4 to 99.2, the mean being 43.5. The lowest specific gravity was 1014, the highest 1035, the mean 1026. To determine whether the concentration of the urine affected the precipitation of calcium oxalate, five specimens of urine containing octahedral crystals were filtered and the filtrates concentrated at a low temperature until the specific gravity reached from 1032 to 1037. In no case was there a further precipitation of oxalate crystals.

ON THE FORMATION OF OXALIC ACID IN THE ANIMAL ORGANISM.

The origin and significance of the oxalic acid found in the urine, has formed the subject of much discussion. Since the observations of Prout, it has been recognized that the oxalic acid taken in the food or in drugs may in part reappear unchanged in the urine. Dunlop claims that all the oxalic acid found in the urine is taken into the body in this way. He bases his opinion on the fact that in patients placed on a milk diet, he was unable to recover any oxalic acid from the urine by quantitative analysis. In accordance with this, Gaglio,¹⁸ Bunge,¹⁹ and Burggraefe,²⁰ feeding dogs on meat alone, found no oxalic acid in the urine. But opposed to Dunlop's hypothesis are the experiments of Salkowski and of Auerbach,²¹ who found oxalic acid in the

¹⁸ Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.

¹⁹ Bunge, *Lehrb. d. phys. u. path. Chemie.* Leipzig, 1889.

²⁰ Burggraefe, *Bull. Acad. roy. de med. de Belg.*, 1862, 2 s., v, p. 327.

²¹ Auerbach, *Virchow's Archiv*, 1879, lxxvii, p. 226.

urine of fasting dogs, and those of Wesley Mills,²² who found oxalic acid in the urine of dogs on meat diet. Primavera has reported an instance of a diabetic patient, with oxaluria, who, when placed on a meat diet, still had a heavy deposit of calcium oxalate in the urine. Bearing on this point, the experiments recorded in Tables V, VI and VII were made:

TABLE V.
PATIENTS ON DIET FREE FROM OXALATES.

	Date.	Diet.	Amount of oxalic acid in 24 hours.	Remarks.
Case I		Continuous milk diet, beginning Nov. 10.		
"	Nov. 16	Milk.	None.	
"	Nov. 17	"	"	
"	Nov. 18	"	"	
"	Nov. 19	"	Slight trace.	
"	Nov. 20	Milk and sugar.	None.	
"	Nov. 21	Milk and sugar, 45 grms.	None.	Sediment examined after precipitation by alcohol. No calcium oxalate crystals.
"	Nov. 22	Milk and sugar, 45 grms.	"	Calcium oxalate crystals formed.
"		Mixed diet from Nov. 23-Nov. 30.		
"		Milk diet Dec. 1- Dec. 4.		
"		Milk and sugar, 45 grms. Dec. 5-14.		
"	Dec. 8	Milk and sugar.	3.9 mg.	No calcium oxalate crystals.
"	Dec. 14	" "	1.1 mg.	Calcium oxalate crystals formed.
Case II		Milk.	None.	
"	Nov. 3	"	"	
"	Nov. 4	"	"	
Case III ..		Milk, 1 week.	"	
Case IV ..		Meat, 1 year.	Slight trace.	
Case V ..		Milk and beeftea.	None.	
Case VI ..		Milk.	None.	
Case VII ..		Milk, 4 days.	Trace.	
Case VIII..	June 24	Carbohydrate diet free from oxalates for 1 week.	} 3.4 mg.	Calcium oxalate crystals rather numerous. Ash free from phosphates and sulphates.
" ..	June 25	Same.		Same as above.
Case IX ..	June 30	Same as above.	37.4 mg.	Calcium oxalate crystals large and numerous. Phosphates in ash.
" ..	July 1	" "	25. mg.	Calcium oxalate crystals large and numerous. No impurities in ash.

²² Wesley Mills, *Journ. of Physiology*, 1885, v, p. 231.

TABLE VI.
DOG'S URINE. DIET FREE FROM OXALATES.

Diet.	Vol.	Amount of oxalic acid.	Sediment examined after precipitation with alcohol.
Meat	210 cc.	0	
"	100	0	
"	500	0	
"	180	0	
Glucose, 5 grm. } Cornstarch, 5 grm. } Water, 300 cc. }	250	.004 grm.	Calcium oxalate crystals.
Cornstarch, glucose....	285	.0012 grm.	
Meat	550	0	
"	170	.0024 grm.	
Meat, cane sugar, } 100 grm. 2 days }	150	.00114 "	No crystals in sediment.
Meat, cane sugar	550	.0119 "	Numerous small octahedral crystals.
Meat, 100 grm. sugar..	52	Trace.	Numerous octahedral crystals.
Placed on milk Jan. 3..			
Milk, Jan. 5	650	Trace.	No calcium oxalate crystals.
Milk, Jan. 7	500	.0086 grm.	Numerous calcium oxalate crystals.
Milk, Jan. 10	650	.0053 "	
Same, Jan. 12	1750	Specimen lost.	
" " 14	1550	.0056 grm.	A few octahedral crystals.

TABLE VII.
FASTING DOG. STOPPED FEEDING JANUARY 24.

Date.	Vol.	Oxalic acid.	Date.	Vol.	Oxalic acid.
Jan. 25-28....	No urine.		Feb. 1.....	100 cc.	.0043
Jan. 29.....	60 cc.	.005	Feb. 2.....	None.	
Jan. 30-31....	No urine.		Feb. 3.....	101 cc.	.0014

In almost every case when a patient was placed upon a diet free from oxalates, the oxalic acid disappeared from the urine, or was present in too small quantities to be of any importance. A marked illustration of the reduction of the oxalic acid excretion by giving a diet free from oxalates is shown in the case of a patient of Dr. S. W. Lambert—a man subject to attacks of renal colic. With a mixed diet, including rhubarb, the twenty-four hours' excretion of oxalic acid was 67.5 mg. When for four days on a diet free from oxalic acid it fell to 4.1 mg.

Cases VIII and IX in Table V were in contrast to all others studied, as when placed on a diet free from oxalates (but rich in carbohydrates)

they continued to excrete an amount of oxalic acid which was above the normal. These were patients in the care of Dr. Hallock of Cromwell, Conn. Case VIII was that of an American woman, unmarried, aged 28 years. She suffered from nervous fears. She had some digestive trouble, at times with headache. Case IX was that of an American woman, aged 54 years, a widow. She had a history of nervous prostration followed by a melancholic condition. She had headache, digestive disturbance and poor sleep. These two cases in contrast with all others examined would definitely indicate that oxalic acid was formed in the body.

To determine whether the ingestion of excessive amounts of carbohydrate food would lead to the production of oxaluria, the following experiment was made:

A dog was placed under observation on Nov. 3, 1899. At that time there was a small amount of oxalic acid present in the urine.²³ The dog was placed on a meat diet, and the urine examined Nov. 18, Nov. 21 and Nov. 25 showed an absence of oxalic acid. On the last named date the dog was placed on large amounts of sugar in addition to meat. The animal took the sugar greedily, at times receiving 250 to 300 grms. in a day. For a month the dog showed no symptoms, but gained rapidly in weight. On Nov. 9 there were noted in the urine a few calcium oxalate crystals, but only a few. From that date until Dec. 27 oxalic acid was absent from the urine or, if present, was in very small amount. In the latter part of December there appeared simultaneously a group of symptoms consisting of loss of appetite, vomiting of frothy mucus, intermittent diarrhœa, the absence of free hydrochloric acid in the gastric juice, the presence of organic acids in the urine, and the precipitation of numerous large calcium oxalate crystals in the urine. On Jan. 1, the dog took almost no sugar, and there were again but few calcium oxalate crystals deposited in the urine. On Jan. 3 very large and very numerous crystals were noted, some appearing in masses of imperfectly formed crystals like microscopic calculi. Whenever the symptoms in this case

²³ To discover the presence of very small quantities of calcium oxalate in the urine, 95% alcohol was added (Salkowski, *Zeitschr. f. physiol. Chem.*, 1886) in the amount of one-third the volume of the urine to be examined. The mixture was then set aside for forty-eight hours, until the calcium oxalate, if present, had crystallized out from the solution. The sediment was then collected from the bottom of the beaker, centrifugalized and examined microscopically. This furnishes a more delicate test for oxalic acid than the complicated quantitative method of analysis.

became severe, the dog would refuse to take its food, when the symptoms (including the oxalic acid excretion) gradually became less marked. The dog was kept on rather large quantities of sugar for six months and throughout that time there was an almost continuous excretion of oxalic acid although the animal received a diet of meat and sugar only.

Two other dogs were treated in a similar way with the same result. These observations indicate that oxalic acid is formed in the animal body.

The question where in the animal organism oxalic acid is formed has been much discussed. Neubauer,²⁴ Bouchardat²⁵ and Ellis²⁶ have claimed that fermentative action in the stomach or intestine may lead to the production of oxalic acid. The most of the writers on this subject have believed that the oxalic acid was formed in the tissues as a result of defective oxidation and that it was probably due to a direct nervous action on the cells.

Oxalic acid, as has been noted above, is produced by many of the higher forms of vegetable life. It has also been noted as a product of lower forms, as of *Aspergillus niger*. Zopf²⁷ has described a form of *saccharomyces* (*S. Hansenii*, Zopf), found in cotton-seed meal, which in fermentable carbohydrate solutions produces oxalic acid in place of alcohol.

Experiment I.—With Dog I, noted above, the following experiment was made: On Feb. 9 the dog was fed 400 gm. of meat and 200 gm. of glucose with water. The dog vomited one hour afterwards 260 cc. The vomitus consisted of undigested meat suspended in a watery fluid which contained a considerable amount of mucus. There was no free hydrochloric acid. On adding hydrochloric acid and heating, there was a butyric acid odor given off. This vomited matter was found to contain oxalic acid.²⁸

²⁴ Neubauer, op. cit.

²⁵ Bouchardat, *Annuaire de thérap.*, 1850.

²⁶ Ellis, *Boston Med. & Surg. Jour.*, 1888, cxviii, p. 64.

²⁷ Cited from Baumgarten's *Jahresbericht*, 1889, v, p. 452.

²⁸ The stomach contents were treated with hydrochloric acid and heated over the water-bath 24 hours. They were then filtered, neutralized with ammonia, and rendered slightly acid with acetic acid. A small amount of calcium chloride solution was then added, and 95% alcohol in the amount of one-half the volume of the fluid. The calcium oxalate was precipitated as a finely divided sediment, and only after about two weeks were the characteristic octahedral crystals formed.

Experiment II.—The same dog was given on Feb. 21, 400 grms. of chopped beef and 200 grms. of glucose and the stomach contents removed in $1\frac{1}{2}$ hrs. The result was the same as before. The specimen contained undigested meat and a large amount of stringy mucus. It was acid in reaction, with no free hydrochloric acid. Oxalic acid was present.

Experiment III.—A mixture was prepared in a flask, which contained cane sugar 100 grms., beef ext., 1 gm., water 1000 cc. To this was added 1 cc. of the gastric contents from Experiment I. This was left in the incubator for two days. On examination oxalic acid was found.²⁹

Experiment IV.—Dog II. Began feeding sugar, 100 grms. daily on Feb. 23. On March 9, calcium oxalate crystals were found in the urine. On that date there were given 200 grms. cane sugar, which was vomited in a half hour. This specimen was examined for oxalic acid with negative result. On Apr. 5 the dog was given 100 grms. sugar in 250 cc. of water. This was withdrawn from the stomach in $1\frac{1}{4}$ hrs. The specimen contained meat which was retained in the stomach from an earlier feeding. There was present thick frothy mucus. There was no free hydrochloric acid. Oxalic acid was present.

Experiment V.—On May 11, 1900, there was fed to Dog I 100 grms. of sugar, in 200 cc. of water. This was removed in one hour. The reaction was faintly acid; there was no free hydrochloric acid present; there was found a large amount of thick mucus. Examination for oxalic acid gave a negative result.

Experiment VI.—A portion of the stomach contents obtained in Experiment V was added to a mixture containing sugar 100 grms., beef extract 1 gm., water 500 cc. After fermenting in the incubator for forty-eight hours, oxalic acid was found.

Experiment VII.—A third dog was fed large amounts of sugar daily, beginning Feb. 22. On May 11 the urine was found to contain a

²⁹ In connection with the fermentation experiments described in this paper, control tests for oxalic acid were made upon uninoculated solutions of Liebig's extract of beef and sugar. These were examined by the same method as the gastric contents (see footnote 28). In fifteen specimens, no oxalic acid was found. In two cases, after diligent search, there was discovered a single small calcium oxalate crystal. The examination of mixtures of chopped beef and sugar for oxalic acid gave negative results. Salkowski (*Berl. klin. Wochenschr.*, 1900, xxxvii, p. 434) records the finding of oxalic acid in beef extract by quantitative analysis. The result of our examinations, however, seemed to prove that, if present, it was in too minute a quantity to affect the validity of the conclusions drawn from the fermentation experiments.

heavy deposit of calcium oxalate crystals. This dog was then fed 100 grms. of sugar in 200 grms. of water and the stomach contents removed in an hour. The reaction was acid. There was no free hydrochloric acid present. There was a moderate amount of mucus. No oxalic acid was found.

Experiment VIII.—A portion of the stomach contents obtained in Experiment VII was added to a mixture containing 100 grms. of sugar, 1 gm. of beef extract and 500 cc. of water. After fermenting in the incubator for two days, oxalic acid was found to be present in the mixture.

Experiment IX.—A mixture was prepared in a flask, consisting of sugar, boiled starch, Huntley and Palmer breakfast biscuits, and water. To this was added 1 cc. of the stomach contents of a patient having persistent oxaluria. There had been no free hydrochloric acid in the stomach contents after a test breakfast. After fermenting in the incubator, oxalic acid was found in this mixture.

Experiment X.—The patient mentioned in Experiment IX was placed on hydrochloric acid given after meals. The calcium oxalate crystals disappeared from the urine almost entirely under this treatment. When the hydrochloric acid had been discontinued for three days, an experiment similar to Experiment IX was made, using a portion of the gastric contents as a ferment. No oxalic acid was formed.

Experiment XI.—To a mixture of 50 grms. of sugar, 0.5 gm. of beef extract and 500 cc. of water there was added 1 cc. of the stomach contents of a patient with marked and persistent oxaluria. After fermenting for two days, oxalic acid was found in the mixture.

The absence of hydrochloric acid in the gastric contents after a test meal, is a noticeable feature in the cases in which the gastric contents were examined. In the case of Dog I, a test was made for free hydrochloric acid, an hour after feeding, on Feb. 9, Feb. 21, Apr. 20 and May 11. Each time the result was negative. In the case of Dog II, the test was made on Mar. 9, Apr. 5 and Apr. 6. Each time there was no free hydrochloric acid found. In the case of Dog III, the stomach contents were examined on March 15 and May 11 and showed an absence of free hydrochloric acid.³⁰

³⁰ In making preliminary experiments to discover an organism producing oxalic acid from solutions of sugar and beef extract (sugar 100 g., beef extract, 1 g., water 500 cc.), it was found that in four specimens, inoculated with baker's yeast, oxalic acid was formed, but in other similar experiments it was not produced. These observations are too few to be of any importance other than to suggest that certain unknown conditions may lead to the formation of oxalic acid through the activity of baker's yeast.

In only three cases was there an examination of the stomach contents of patients having persistent oxaluria. The patient mentioned in Experiment IX had repeated examinations made after test meals, with a constant absence of free hydrochloric acid. The patient mentioned in Experiment XI had long standing oxaluria with local irritative symptoms in the urinary tract. In this case there was no free hydrochloric acid in the gastric juice, and the same result was obtained in a third case with persistent calcium oxalate deposit in the urine.

PHYSIOLOGICAL AND TOXIC ACTION OF OXALIC ACID.

As the presence of oxalate of lime in the urine is found associated with many symptoms, the question naturally arises as to what is the physiological action of oxalic acid and soluble oxalates. Do they produce any of the symptoms of the so-called oxalic-acid diathesis?

Death from oxalic-acid poisoning is ordinarily due to the local corrosive action on the alimentary canal, and large doses may be taken with impunity if they are in well diluted solution. Christison and Coindet³¹ say that early in the century oxalic acid was used extensively in making lemonade, and was generally believed to be innocuous. Piotrowski,³² in experimenting upon himself, took at several different times from 4 to 7 grammes in twenty-four hours with no noteworthy symptoms, and once he took 8 grammes within one hour.

With a view to studying the influence of rather large doses of soluble oxalates, extending over a number of days, the following experiments were made. A healthy man received a diet free from oxalates and was given daily for two weeks from .20 to .50 gm. of ammonium oxalate. This was taken well diluted immediately after meals. The oxalate was given at first alone, then with enough sodium bicarbonate to render the urine but faintly acid, then with dilute hydrochloric acid. The alkali and acid were given to determine the influence of the acidity of the gastric juice upon the absorption of the drug. Table VIII shows the result of the experiment.

³¹ Christison and Coindet, *Edinb. Med. & Surg. Jour.*, 1823, xix, pp. 163; 323.

³² Bucheim, *Arch. d. Heilk.*, 1857, n. F., i, p. 124.

The same experiment was carried out upon another subject with the result shown in Table IX (p. 42).

In the first of these two cases there was no symptom noted excepting polyuria, as indicated in the table. In the second case no symptom whatever followed the ingestion of the drug. In neither case was the excretion of oxalic acid increased above the normal.

TABLE VIII.

HEALTHY MAN. DIET FREE FROM OXALATES. GIVEN AMMONIUM OXALATE.

Date.	Diet.	Sp. gr.	Reaction.	Volume of urine.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid in urine.	Sediment after precipitation with alcohol.
Jan. 20	Beef, rice	1016	Acid.	1260	15.4	.359	42.9	None.	None.	.0055	Octahedral crystals abundant.
21	Milk 1200 cc., butter	1016	"	1545	23.	.487	47.2	"	"	.002	" " small.
22	Huntley & Palmer's biscuits	1012	"	2035	23.5	.436	53.7	.225 grm.	"	.0059	" " "
23	" " " "	1014	"	2045	30.	.684	43.8	.35	"	.0065	" " "
24	2 lamb chops, 2 eggs	1010	"	2410	25.9	.103	25.1	.425	"	.001	" " numerous.
25	As at first.	1017	Family acid.	1565	25.4	.335	75.8	.15	"	.014	Calcium sulphate crystals; small octahedral crystals.
26	" " " " " "	1020	"	1280	24.8	.324	76.6	.45	"	.0016	Octahedral crystals numerous.
27	" " " " " "	1016	"	2105	28.1	.566	49.7	.45	"	.0051	" " small.
28	" " " " " "	1013	"	2680	25.5	.407	62.8	.475	"	.024	" " few.
29	" " " " " "	1014	"	2160	31.1	.564	55.	.375 Sod. bicarb. 4.2 grm.	"	.002	" " "
30	" " " " " "	1013	"	2405	30.8	.515	59.9	.375	4.8	.019	" " very few.
31	" " " " " "	1015	"	1975	23.3	.444	52.5	.375	3.6	.036	" " numerous.
Feb 1	" " " " " "	1010	"	2400	37.4	.513	72.8	.375	3.6	.0038	" " few.
2	" " " " " "	1013	"	2530	34.43	.502	61.3	.350 HCl 3.6 cc.	"	.0057	No octahedral crystals.
3	" " " " " "	1010	"	2295	33.9	.437	77.6	.44	5.4 cc.	.015	Few " "
4	" " " " " "	1012	"	2370	29.8	.157	190.	.30	5.4	.013	Very few " "

Two dogs were given ammonium oxalate, in larger doses, in proportion to body weight, than the men received. The results of these experiments are recorded in Table X (p. 43).

This dog developed no symptoms excepting albuminuria which disappeared whenever the ammonium oxalate was withheld and reappeared whenever it was given. After receiving the dose intraperitoneally, it developed peritonitis and died within a few hours.

Another dog was given ammonium oxalate subcutaneously. On Feb. 28, .5 gm. of ammonium oxalate was given hypodermatically in 100 cc. of water. The dog showed no symptoms for twenty-four hours when there was noted a weakness in the hind extremities. At that time .5 gm. was again injected. There were no symptoms noted excepting increasing loss of power in the extremities until Mar. 4, when the respiration became rapid and panting and the animal died. After the first

TABLE IX.
HEALTHY MAN. GIVEN AMMONIUM OXALATE.
Diet—Bread, butter and meat.

Date.	Amount in 24 hours	Reaction.	Sp. Gr.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid secreted.	Micros. Exam.
Feb. 23	1120 cc.	Acid.	1017	13.48	.6237	21.61	0	0	.00502	No oxalate crystals.
24	945	Faintly acid.	1021	17.32	.6294	27.51	0	0	.00846	Small oxalate crystals.
25	1585	Faintly acid.	1017	25.11	.5016	50.06	.24	0	.0071	Numerous oxalate crystals
26	715	Acid.	1021	14.76	.4706	31.36	.28	0	.0064	" " "
27	815	Acid.	1025	19.18	.3523	54.44	.30	0	.0112	" " "
28	760	Markedly acid.	1022	19.24	.3517	54.91	.30	0	.0007	" " "
Mar. 1	1400	Strongly acid.	1018	14.33	.4199	34.13	.30	Sod. bicarb.	.0067	" " "
2	995	Very faintly acid.	1019	18.58	.5543	33.52	.30	Sod. bicarb.	.0229	Octahedral crystals fewer.
3	945	Very faintly acid.	1019	20.03	.5556	35.81	.38	Sod. bicarb.	.0030	[numerous.
4	1740	Very faintly acid.	1016	25.23	.7387	34.22	.42	Sod. bicarb. HCl	.0179	Octahedral crystals fairly
5	1165	Faintly acid.	1018	18.62	.3132	59.48	.42	5.4 cc. HCl	.0074	Trace of sulphates left.
6	2100	Faintly acid.	1009	15.45	.9076	17.02	.48	5.4 cc. HCl	.0087	Numerous cal. oxal. crystals and cal. sulphate.
								5.4 cc.		Calcium oxalate crystals not very numerous.
										Very few calcium oxalate crystals.

injection of ammonium oxalate there was no urine voided for forty-eight hours. Then 220 cc. were passed. This contained no albumin, no sugar nor other reducing agent. .1466 gm. of oxalic acid was recovered. The next day 250 cc. of urine were passed, containing a trace of albumin, no sugar or other reducing agent. There was found .078 gm. of oxalic acid. On the next day 690 cc. were voided containing .057 gm. of oxalic acid. There was thus given in all, subcutaneously, 1 gm. of ammonium oxalate, which represents .726 gm. oxalic acid. Of this there was recovered in the urine .281 gm. or 37%.

The autopsy on this dog was made one-half hour after death. The blood was still fluid throughout the body, but it slowly coagulated upon exposure to the air. The heart was in diastole, filled with liquid blood,

with a single small clot in the left ventricle. The lungs showed areas of congestion. The most interesting lesion was in the kidney. Here the section showed numerous crystals blocking up the uriniferous tubules. Most of them were of irregularly shaped masses. Some showed characteristic dumbbell and ovoid shapes. These crystals were of a very light yellow color, were unstained by eosin or hæmatoxylin, were insoluble in acetic acid and ammonia, but dissolved in dilute hydrochloric acid. There were no changes in the glomeruli. The cells lining the tubules were in places swollen and granular, and in places were torn away by the passing of a calculus.

TABLE X.
ADMINISTRATION OF AMMONIUM OXALATE TO DOG.

Date.	Amount of urine.	Albumin.	Ammon. oxalate taken.	Oxalic acid taken in form of ammon. oxalate.	Amount of oxalic acid excreted.
Feb. 4...	144 cc.	None.	.50 grm.	.36	.0285
Feb. 5....		"	.50 grm.	.36	
Feb. 6....		"	0	0	
Feb. 7... }	315 cc.	"	.75	.54	Specimen lost.
" 8.... }		"	1.00	.72	
" 9.... }		"	1.00	.72	
" 10.... }	175 cc.	"	1.00	.72	.0242
" 11.... }		"	1.00	.72	
" 12.... }		Trace.	1.00	.72	
" 13.... }	565 cc.	None.	1.00	.72	.0707
" 14.... }			0	0	
" 15.... }			0	0	
" 16.... }	90 cc.	Trace.	.4	.29	.0035
" 17.... }			.4	.29	
" 18.... }			0	0	
" 19.... }	980 cc.	None.	0	0	.0034
" 20.... }			0	0	
" 21.... }			0	0	
" 22.... }	110 cc.	Large amount.	0	0	
" 23.... }			0	0	
" 24.... }			1 grm.	.72	
" 25.... }			intraperitoneally.		

The study of acute oxalic acid poisoning does not come within the scope of this paper. It may, however, be noted, in connection with the above experiments, that the symptoms of the acute poisoning have been carefully studied by Christison and Coindet,³³ R. Koch,³⁴

³³Op. cit.

³⁴R. Koch, *Arch. f. exp. Path. u. Pharm.*, 1881, xiv, p. 153.

Kobert and Küssner,³⁵ and Krohl,³⁶ and the pathology has been studied by A. Fraenkel³⁷ and by Ebstein and Nicolaier.³⁸ The most common symptoms are those of the nervous system: either irritative phenomena, as muscular twitchings and tonic or clonic convulsions; or paralytic phenomena—paresis or paralysis, both motor or sensory, with abolition of reflexes, and excessive lowering of temperature before death. The pulse, respiration and blood-pressure are not influenced except in fatal cases, and there are no digestive symptoms unless the drug is given by mouth. Several observers have found in the urine a strong reducing agent, acting upon copper sulphate and bismuth subnitrate, which does not give the test for sugar by the spectroscope or polariscope. The urine may contain albumin or casts or red corpuscles or any of the forms of calcium oxalate crystals.

On the Oxidation of Oxalic Acid in the Organism.—The slight danger in taking large doses of soluble oxalates seems to be due in part to the precipitation of the oxalic acid as calcium oxalate in the alimentary canal, and its remaining unabsorbed in the fæces; and in part to the oxidation in the organism of that which is absorbed. Gaglio,³⁹ experimenting with a cock, recovered from the fæces all the oxalic acid that was taken; but Guinti,⁴⁰ while verifying Gaglio's experiment in the case of the cock, found that in dogs and man, a portion of that absorbed was oxidized.⁴¹

In the experiments described above it will be noted that in the cases of the men receiving ammonium oxalate, in amounts varying from .2 gm. to .48, and extending over a period of eleven days in

³⁵ Kobert and Küssner, *Virchow's Archiv*, 1879, lxxviii, p. 209, and 1880, lxxxi, p. 383.

³⁶ Krohl, *Arch. a. d. pharmakol. Inst. z. Dorpat*, 1891, vii, p. 130.

³⁷ A. Fraenkel, *Zeitschr. f. klin. Med.* 1881, ii, p. 664.

³⁸ Ebstein and Nicolaier, *Virchow's Archiv*, 1897, cxlviii, p. 366.

³⁹ Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.

⁴⁰ Guinti, *Ann. di chim. e di farm.*, Milan, 1897.

⁴¹ Thus in giving a man .31 gm. he recovered in the fæces .038 gm., leaving .272 absorbed. Of this there was excreted in the urine during the first two days .062 gm. or 11%. From this he concludes that the rest was oxidized in the system. In a similar experiment on a dog he recovered 11% of that absorbed. But in giving the drug hypodermatically to a dog, he recovered at one time 43.6%, and again 51% of that injected.

one case, and two weeks in the other, only traces of oxalic acid appeared in the urine, while in the case of the dog receiving the drug subcutaneously 37 per cent was recovered. As no study was made of the faeces in these experiments it is impossible to form any opinion as to whether any oxalic acid was oxidized in the body.

CONCLUSIONS.

1. As varying amounts of calcium oxalate may be held in solution in the urine, conclusions based upon the presence or number of calcium oxalate crystals found therein are of no real value as an indication of the quantity of oxalic acid present.

2. Unless the utmost care is exercised, the results obtained by quantitative estimation of oxalic acid are subject to large percentages of error. This is especially true in the use of Neubauer's or Shultzen's methods, in which the calcium oxalate is precipitated in an alkaline solution.

3. An ordinary mixed diet regularly contains traces of oxalic acid or its salts.

4. A portion of the oxalic acid ingested with the food may be absorbed and reappear unchanged in the urine.

5. The normal daily excretion of oxalic acid in the urine fluctuates with the amount taken in the food, and varies from a few milligrammes to two or three centigrammes, being usually below ten milligrammes.

6. In health, no oxalic acid, or only a trace, is formed in the body, but that present in the urine has been ingested with the food.

7. In certain clinical disturbances which in some of the cases studied above were associated with absence of free hydrochloric acid from the gastric juice, oxalic acid is formed in the organism.

8. This formation in the organism is connected with fermentative activity in the alimentary canal.

(a) The prolonged feeding of dogs with excessive quantities of glucose, together with meat, leads eventually to a state of oxaluria.

(b) This experimental oxaluria is associated with a mucous gastritis, and with absence of free hydrochloric acid in the gastric contents.

(c) The oxaluria and the accompanying gastritis are referable to fermentation induced by the excessive feeding with sugar.

(d) The experimental gastritis from fermentation is associated with the formation of oxalic acid in the gastric contents.

9. The symptoms attributed to an oxalic acid diathesis, with the exception of those due to local irritation in the genitourinary tract, do not appear to be due to the presence in the system of soluble oxalates, but are more likely to depend on other products of fermentation and putrefaction.

I wish to express my gratitude to Dr. Herter, at whose suggestion and under whose guidance this study has been made, and to Dr. A. J. Wakeman for advice and aid in carrying on the work.

SERUM-GLOBULIN AND DIPHTHERIC ANTITOXIN.—A
COMPARATIVE STUDY OF THE AMOUNT OF GLOBU-
LIN IN NORMAL AND ANTITOXIC SERA, AND THE
RELATION OF THE GLOBULINS TO THE ANTITOXIC
BODIES.¹

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The varying statements concerning the amount of globulin in serum from immunized and non-immunized horses, and concerning the relation of the antitoxic principle to this constituent and to other constituents of serum, leave no doubt as to the confusion existing in regard to these questions, and indicate a promising field of research.

The propositions upon which our work was primarily based were the following: To analyze normal sera and antitoxic sera of different values for the determination and comparison of the amounts of globulin and albumin, and of the antitoxic values of these globulins and albumins, and also of the various filtrates accompanying their preparation.

In the analysis of blood-serum various methods have been devised and recommended for obtaining the different ingredients of the serum in a pure state. Of these methods, those recommended for the precipitation of the globulins are of chief interest to us and will be briefly considered. The principal methods are the following: (a)

¹ Received for publication February, 1900. The experiments recorded in this paper were begun in May, 1897, and have extended over the intervening years. They have been performed at the Research Laboratory of the Department of Health of New York City, where exceptional opportunities for such investigations exist, owing to the large number of horses constantly undergoing immunization for the production of diphtheric antitoxin.

precipitation of the globulins by super-saturation of the serum with magnesium sulphate or semi-saturation with ammonium sulphate; (b) precipitation by means of CO_2 gas, by passing it through solutions containing the serum or globulins; and, (c) finally, precipitation by dialysis, this method depending for its success upon the removal of the natural or added salts, the globulins being looked upon as insoluble in pure water and hence precipitated by removal of the salts.

Dieudonné² has shown that marked differences exist in the action of the globulins prepared by different methods, when tested for their antitoxic power. Globulins obtained by precipitation with CO_2 , according to him, display the least antitoxic value; those obtained by precipitation with magnesium sulphate the greatest; while globulins, the product of dialysis, hold an intermediate place in the antitoxic scale.

In his experiments, Dieudonné used a normal horse's serum which, he says, showed marked antitoxic properties. One cubic centimetre of this serum mixed with twice the fatal dose of diphtheric toxin, *in vitro*, protected the animal even from marked local symptoms. Globulin prepared from this serum by CO_2 gas was found to have practically no antitoxic value. This result differed so widely from the results claimed by Smirnow,³ that another experiment was undertaken in which the globulins were precipitated by the addition to the serum of an excess of magnesium sulphate. This was the method that Smirnow had employed, and Dieudonné found "the preparation obtained by the magnesium sulphate acted very differently from that prepared by carbonic acid; it showed toxin-neutralizing properties like those obtained by Smirnow in his experiments with globulin." Further experiments brought to light the fact that globulins won by means of dialysis were markedly less active than the precipitate obtained by magnesium sulphate, but that the filtrate had a noticeable antitoxic value.

Dieudonné concludes from his experiments "that the antitoxic property does not belong to the globulin, but to some unknown body contained in the serum which in the preparation of the globulin is carried down mechanically in the precipitate, or becomes closely bound up with it; and that by the rapid precipitation with magnesium sulphate this

² *Arch. a. d. k. Gesundheitsamte*, 1897, xiii, p. 297.

³ *Arch. d. sciences biol.*, St. Pétersb., 1895, iv, p. 328.

active substance is much more easily seized, than in the slower separating out of the globulins by carbonic acid or by dialysis, in which only a small portion is taken with the precipitate." This active body contained in serum must therefore, he concludes, be least precipitable by carbonic acid, somewhat more precipitable by dialysis, and most precipitable by magnesium sulphate; and hence the action of the various filtrates obtained in the different methods of globulin preparation is also different. After precipitation by magnesium sulphate these fluids are poorest in antitoxic bodies; after carbonic acid preparation, richest. This becomes especially apparent if the following experiments of Dieudonné are considered.

The precipitate previously obtained by magnesium sulphate was redissolved and subjected to dialysis in flowing and distilled water. The precipitate thus obtained by dialysis was redissolved in 2% salt solution. The original magnesium sulphate globulin precipitate had a high protective value, while even 2 cc. of the fluid remaining after the abstraction of the globulin was totally inactive. These proportions were very different after dialysis. The globulin precipitated by dialysis was only weakly active, while of the fluid freed from the globulin, 0.5 ccm. still displayed marked neutralizing powers. These differences are more apparent still in the second experiment, *i. e.*, the purification of the magnesium sulphate globulin by means of carbonic acid gas. One gramme of the dried magnesium sulphate globulin was dissolved in 50 cc. of water. In passing CO₂ gas directly into this solution not a trace of precipitate was to be noted. The solution was therefore subjected to dialysis, first in running water, then in distilled water, until no trace of sulphuric acid could be determined. In the parchment tube an abundant precipitate appeared, which was redissolved in 2% sodium chloride solution. Into this solution carbonic acid gas was run for two hours and a fine flocculent precipitate obtained. In testing the antitoxic value of this precipitate it was found to have lost its value and that the antitoxin was for the most part in the filtrate instead of with the globulin as in the original magnesium sulphate precipitation.

These experiments have seemed worth detailing in this connection since an explanation of the results obtained has been advanced by Seng⁴ in his recent paper. Seng mentions that Brieger's⁵ observation that the antitoxins from zinc chloride precipitation go over into the filtrate from reprecipitation with carbon dioxide, while those from zinc sulphate

⁴ *Zeitschr. f. Hygiene*, 1899, xxxi, p. 513.

⁵ *Ibid.*, 1896, xxi, p. 267.

precipitation remain with the precipitate when reprecipitation by carbon dioxide is practiced, demonstrates that the chemical nature of the resulting precipitate or of the precipitating method decides whether the antitoxin falls with the precipitate, but shows that it is in no wise carried down by the precipitate.

He cites experiments in support of this view and against Dieudonné's conclusion that the antitoxins are mechanically carried down with the precipitates, and says: "That a further weighty support to this view was brought by the researches of Dr. Sternberg," who, finding that "it was inconvenient to work with such richly albuminous antitoxin solutions as those composing the native diphtheric serum," was able to show "that the albumin could be removed by other than the Brieger method from the antitoxin, and that by the addition of $\frac{1}{3}$ vol. of a 5% (concentrated) solution of potash alum to the serum, a great part of the albuminous bodies would be precipitated as an abundant, voluminous, precipitate, the antitoxins, however, remaining in solution."

Here, then, the antitoxins remained in solution in spite of a voluminous precipitate, and by further experiments it was shown that no matter whether the albumins were previously removed or whether the globulins were precipitated by magnesium sulphate or ammonium sulphate, nevertheless, the entire antitoxin always remained with the globulins.

The vital point in Seng's experiments was reached in removing the excess of the precipitating salts from the globulins. The removal of the salts was accomplished by dialysis, and it was to be expected, according to previous conceptions, that, by a dialysis which was carried on until all traces of chlorine, ammonia, and sulphuric acid reaction had disappeared, all globulins would be precipitated in the salt-free solution, but it appeared that only a very small part, 1:23 to 1:11, of all the globulin separated itself out as insoluble, the majority of the globulin and with it the entire antitoxin remaining in solution. It was determined by experiment that the antitoxins clung to these "soluble globulins."

This may be the explanation of the discrepancies noted when globulins obtained by different methods are tested for their antitoxic value, the antitoxin remaining apparently with the "soluble globulins" only. Both the soluble and the insoluble globulins are precipitated by magnesium sulphate, but only the insoluble by dialysis.

Early in our own work it became apparent that the magnesium sulphate method was the most trustworthy known to us for obtaining

globulins and the antitoxic principles of the serum. Throughout the paper, therefore, the results of precipitation by magnesium sulphate will alone be given.

In obtaining the globulin content of the various sera, both normal and antitoxic, the following method has been followed:

Separation of the globulins from blood-serum.—10 cc. or 20 cc. of the serum was diluted to 50 cc. or 60 cc. with a saturated solution of magnesium sulphate. Crystals of magnesium sulphate were then added and the mixture stirred until completely saturated. When precipitation was complete, the precipitate was filtered out and redissolved in water together with the crystals of magnesium sulphate still remaining in the original beaker glass, the quantity of solution thus resulting amounting generally to 350 cc. to 400 cc. From this solution the globulin was again precipitated with magnesium sulphate and filtered out, the beaker being washed with a saturated solution of magnesium sulphate, which fluid was then poured onto the filter. The globulin thus obtained was washed on the filter paper with a saturated solution of magnesium sulphate, and finally redissolved in distilled water.

These precipitates were then tested upon guinea-pigs in order to determine their antitoxic values and to compare these with the values of the sera from which they were derived.

All tests of serum for antitoxic value were made by mixing the toxin and serum and then injecting the mixture into the test animal.

Determination of the antitoxic value of the globulins.—In making the tests for antitoxin in globulin solutions, exactly the same method was followed as that employed in testing serum itself. The globulin from the 10 cc. or 20 cc. of the serum was dissolved in a known quantity of sterile distilled water. Of this solution a quantity corresponding in globulin content to 1 cc. of the serum, from which it was derived, was diluted to the required strength and mixed as usual with the 10-times fatal dose of toxin.

Table I shows the results of the tests with globulins. Some tests of albumins from the sera are recorded in Table II.

Toxin control animals for all these tests died in the usual time, 4 to 7 days.

From Tables I and II it can be readily seen that the tests of the globulins obtained by magnesium sulphate precipitation show that practically the total antitoxin content of the various sera is associated

with the globulins, these globulins showing an antitoxic value equal to the strength of the antitoxin contained in the serum from which they were derived.

TABLE I.
DETERMINATIONS OF ANTITOXIC VALUE OF GLOBULINS FROM ANTITOXIC SERA.

Date.	No. of Horse.	Units of Anti-toxin in 1 ccm. of serum.	Units of Anti-toxin tested for in glob. from 1 ccm. of serum.	Weight of test Guinea Pig.	Result of Test.
26-III-'97 ..	83	500	500	278	Animal not affected.
30-XII-'97..	105	500	500	270	" " "
1-XII-'97..	112	550	550	230	" " "
1 " '97..	112	550	550	228	" " "
1 " '97..	112 ⁶	550	550	260	" " "
1 " '97..	112 ⁷	550	550	237	" " "
3-VI-'97 ..	128	500	500	218	" " "

TABLE II.
DETERMINATIONS OF ANTITOXIC VALUE OF ALBUMINS.

Date.	Horse.	Units of Anti-toxin in 1 ccm. of serum.	Units of Anti-toxin tested for in Albumin from 1 ccm. of serum.	Weight of test Guinea Pig.	Result of Test.
26-III-'97 ..	83	500	5	222	Animal died in 5½ days.
3-VI-'97 ..	128	500	5	272	Animal died in 1½ days.

To determine the action of magnesium sulphate by itself upon the toxins, and thus rule out any error that might arise from such action, if present, the following experiment was undertaken:

A solution of magnesium sulphate calculated to be the same in strength as would be found with solutions of the globulins was mixed with toxin as follows and injected into guinea-pigs:

TABLE III.
TO DETERMINE ACTION OF MAGNESIUM SULPHATE ON TOXIN.

Weight of test Guinea Pig.	ccm. of Mg SO ₄ sol.	Fatal doses of toxin.	Result of test.
225 gm.	2 ccm.	2	Died in 3 days.
" "	2 "	4	" " 2½ days.
" "	2 "	6	" " 2¼ days.

⁶ Same globulin solution as 112, but kept exposed to light at room temperature in stoppered blue-glass bottle for 2½ months.

⁷ Same globulin solution as 112, but kept in bottle in ice-chest three months before testing.

As is evident from Table III, no neutralizing action upon the toxin was determined, the test animals dying in about the usual time; we may also conclude from these experiments that the Mg SO_4 had no stimulating effect upon the animal.

To determine, on the other hand, the evil effects of injecting Mg SO_4 into guinea-pigs the tests recorded in Table IV were made:

TABLE IV.
TO DETERMINE INJURIOUS EFFECTS OF INJECTION OF MAGNESIUM SULPHATE.

Weight of test Guinea Pig.	ccm. of sat Mg SO_4 sol.	Result of test.
300 gm.	0.5 ccm.	Animal not affected.
335 "	0.25 ccm.	" " "
307 "	0.1 ccm.	" " "
250 "	1 ccm.	" died in 10 minutes.

As the globulin from 1 cc. of an antitoxic serum of 500 units strength, when tested for this strength, is diluted 5000 times, it is plain from the above results that the amount of magnesium sulphate injected into an animal with the globulin solution must be too small in quantity to have any appreciable effect on the animal.

Having, by the foregoing experiments, demonstrated to our satisfaction that the magnesium sulphate precipitates contained practically all the antitoxic bodies—be these globulins or bodies precipitated with them in the presence of magnesium sulphate—we were then in a position to carry on the major part of our work, *i. e.* the comparison of the weights of these precipitates as obtained from the serum of non-immunized and of immunized horses at various stages of resistance. These determinations were made in the manner detailed below, the greatest care being taken to avoid error, several determinations often being made from the same serum.

Coagulation and weighing of the total coagulable albuminous bodies (proteids); globulins, and albumins.—The globulin and albumin were coagulated together from the horse serum in the following manner: 10 cc. or 20 cc. of the serum, as the case might be, were diluted to 350 cc. or 400 cc. with water, and the temperature gently raised over the Bunsen burner until coagulation began. The temperature was then quickly raised to boiling point, the solution being constantly stirred. When

the solution was boiling well, dilute acetic acid was added drop by drop until a faint acidity was reached. After a few moments more of boiling, the coagulation was complete and the flocculent mass quickly settled to the bottom of the beaker glass. The coagulated proteids were filtered onto a weighed filter paper, washed with boiling water, and, finally, slowly dried in the air bath to constant weight.

The globulin obtained by precipitation with Mg SO_4 was coagulated and filtered from its watery solution in precisely the same manner. The difference between the weight of the globulin and the total albuminous (proteid) precipitate was considered as the weight of the albumin in the serum taken. This method is open to some objections, but with careful technique very accurate results for comparison may be obtained.

Table V gives the results of these experiments. The determinations are given in grammes.

A consideration of the figures of Table V convinces us that although there is no absolute conformity in the amount of the precipitates from sera of the same antitoxic value, still even in the comparison of these sera from different horses it is apparent that the lower the antitoxic value of the serum the lower is the globulin content, *i. e.* if normal serum, according to our determination for 20 ccm., gives 0.8 gm. of precipitate, then 200-units serum will average much higher, 300 higher still, and so on, if a long series is taken. But there were such marked exceptions to this rule that it was evident that a high antitoxic value is possible in a serum of comparatively low globulin content, as low often as a serum of practically no antitoxic value. The absolute amount was then no index of antitoxic value. This dispelled our hope of determining before undertaking immunization the value of a horse for the production of antitoxin by globulin determinations.

It early became evident, except for the interest of general statistics and comparison, that determinations based upon the sera of different horses were of little value, and that the only safe data upon which to found conclusions would be those obtained from the serum of the same animal, from the normal state on to a high degree of immunity. In several cases it has been possible to make such determinations.

From Table VI it is obvious that, no matter what the initial globulin content, this amount was always increased as the immunization

TABLE V.
DETERMINATION OF TOTAL ALBUMINOUS BODIES (PROTEIDS)—GLOBULINS AND
ALBUMINS—FROM SERA OF DIFFERENT ANTITOXIC VALUES.

Date.	Horse.	ccm. of serum used.	Antitoxic units per ccm.	Weight of globulin.	Estimated wt. of albumin.	Weight of albuminous bodies (glob. and alb.) precipitated by heat.
16-XI-'97.	7	20	300	1.4077	.4928	1.9005
18-XII-'97.	"	"	400	1.5548	.5194	2.0742
11-V-'97.	83	"	200	.9834		
29-VI-'97.	"	"	400	1.3344	.2354	1.5698
?	"	"	500	1.4683	.3959	1.9642
6-XII-'97.	"	"	850	1.5298	.4695	1.9993
?	89	"	Normal.	.5710	.4707	1.0417
21-V-'97.	91	"	200	.7358	.3263	1.0621
13-IV-'97.	"	"	300	1.1720	.3928	1.5648
?	95	"	Normal.	.7265	.6465	1.3730
8-XII-'97.	96	"	300	1.3540	.4123	1.7663
29-XI-'97.	"	"	400	1.5949	.5143	2.0806
27-IX-'97.	98	"	Normal.	.6171	.8057	1.4228
?	99	"	"	.7593		
?	100	"	"	.8150	.5610	1.3760
?	101	"	"	.7602		
?	101	"	200	1.4193		
?	103	"	Normal.	.8685	.6535	1.5220
21-IV-'98.	108	"	"	.6743	.6871	1.3614
	"	"	200	.8436		
?	109	"	Normal.	.5818	.8376	1.4194
10-XII-'98.	110	"	"	.7559	.6834	1.4393
	110	"	300	1.2101	.5773	1.7874
27-XI-'98.	111	"	Normal.	.6377	.8221	1.4598
	"	"	500	1.5447	.2455	1.8902
	112	"	450	.7569	.7281	1.4850
	"	"	500	1.0591	.5918	1.6509
	116	"	Normal.	.7977		
	117	"	"	.9943	.6826	1.6819
	118	"	"	.7773	.7373	1.5146
	119	"	"	.7673	.6532	1.5205
	"	"	150	.8056	.5908	1.4964
	121	"	Normal.	1.1429	.5944	1.7373

proceeded, although no fixed amount of increase seemed to represent a given number of units of antitoxic value. The same thing is apparent in Table VII, where lists are given of determinations from horses in whose case it was impossible to obtain data concerning the normal serum.

TABLE VI.
GLOBULIN DETERMINATIONS FROM NORMAL AND ANTITOXIC SERA.⁸

		Units of Antitoxic Strength.					
		Normal.	150	200	300	400	500
Horse	89.....	.5710					
"	95.....	.7265					
"	98.....	.6171					
"	99.....	.7593					
"	100.....	.8150					
"	101.....	.7602		1.4193			
"	103.....	.8685					
"	108.....	.6743		.8436			
"	109.....	.5818					
"	110.....	.7559			1.2101		
"	111.....	.6377					1.5447
"	116.....	.7977					
"	118.....	.7773					
"	119.....	.7673	.8056				
"	121.....	1.1429					

We again find demonstrated the excess of the magnesium sulphate precipitates from the sera of horses which have reached high degrees of immunization over the sera of the same animals at a less advanced stage.

TABLE VII.
GLOBULIN DETERMINATIONS FROM ANTITOXIC SERA.⁹

		200	300	400	450	500	550	600	650	700	750	800	850
Horse	7		1.4077	1.5548									
"	83			1.3344		1.4683							1.5297
"	91	.7358	1.1720										
"	96		1.3540	1.5949									
"	112				.7569	1.0591							

In Table VIII are given some globulin and albumin determinations made from 10 cc. amounts of various sera. The globulins were pre-

⁸ Determinations made from 20 cc. of serum, and results given in grammes.

⁹ Determinations made from 20 cc. of serum, and results given in grammes.

cipitated with $Mg SO_4$ in the regular manner; and the albumins were then precipitated from the filtrate by heat. The precipitates were collected, however, on Gooch crucibles instead of on filter-papers, and the weighings thus made with probably less error than in the methods used by us earlier in making these determinations. Nevertheless, remembering that we are dealing with 10 cc. instead of 20 cc. amounts of serum, we find the results practically unaltered. It is interesting to note the decrease shown by the albumins as the globulins increase. Whether this is a coincidence, or a regular gain of the one at the expense of the other, it would be difficult to determine from our few tests. If it is a constant occurrence, it may be that a transmutation takes place, or simply that the process which regulates the albumin content is depressed during the period of exaltation of globulin production.

TABLE VIII.

GLOBULINS.

Horse.	Normal.	400	500	600	650	1200
1333727	.7782				
13538648987			
13635667389		
1373235	.47435116	.5934	.8987

N. B.—Horse No. 137 went from normal to 1200 units at first bleeding; subsequent bleedings with no intervening toxin inoculation showed constant decrease in the number of units, likewise in globulin.

ALBUMINS.

1334146	.2102				
13536802341			
13636182715		
1373127	.31332938	.2727	.2341

TABLE IX.

GLOBULIN DETERMINATIONS FROM SERUM OF HORSE 83.

	200	300	400	500	850
Horse 83.....	.9834				
“ “9666	This serum dropped from 700 units.		
“ “	1.0218	“ “	“ “	500 “
“ “	1.3344		
“ “	1.1478	This serum dropped from 600 units.	
“ “	1.4683	
“ “	1.5297
Average.	.9750	1.0218	1.2411	1.4683	1.5297

The comparison given in Table IX is exceedingly interesting. The table shows the determinations from one of the horses that has long been a source of high grade antitoxin. Figures are given for the magnesium sulphate precipitates from 200 unit serum to that containing 850 units, with the difference of 0.5 gm. between the determination of 200 unit serum and the 850 unit specimen.

The most interesting point in this connection is that some of these specimens when first drawn and tested gave a high number of units, and that, after having been kept, they dropped very considerably in their antitoxic value and globulin content, this being due possibly to some obscure chemical change or more probably to contamination with microorganisms. Comparing two 200-unit specimens, one of which had dropped from 700 units, and the other having been freshly drawn, we find their globulin content practically the same. A 400-unit specimen, which had dropped from 600 units, showed a like decrease in the amount of globulin; likewise a 500-unit serum, which had fallen to 300 units, gave a precipitate in weight between that of the 200 and 400-unit specimens.

Although we do not feel justified in positively concluding from the foregoing facts that the antitoxin, or antitoxic bodies as we prefer to call them, are globulins, yet we cannot help feeling that such facts point very strongly to their being globulins, or at least bodies which have in all points, in the light of our present knowledge, the same reactions as the globulins. It seems a remarkable coincidence that the antitoxic bodies and the globulins should have been destroyed in such exact proportion in those sera which had lost in antitoxic value, if they were not the same bodies.

Undoubtedly included under the general term "serum globulin" there are several or many bodies of slightly varying chemical characters that eventually we may be able to distinguish one from the other, and determine their absolute amounts. Some of these globulins, it seems not unlikely, may, at times, be "cell globulins" in contradistinction to the true "serum globulins," and be found in the serum after some unusual destruction of the formed elements of the blood.

The variations in the initial amounts of globulin present in the serum of different animals may be due to normal variations in the physiological processes, but many of them are possibly to be explained as pathological conditions, and among these may be increases due to various past infections through which the animal has successfully passed, or to minor infections, less obvious, but existing before and at the time of the taking of the specimen for analysis. All such complicating conditions should be taken into consideration, and an attempt made to reduce results to uniformity by excluding such complications or allowing for their existence when making comparisons.

In considering initial amounts of globulin the antitoxic value of so-called "normal serum" in each case should be carefully determined, as a partial explanation of variations in initial amounts of globulin may be found in the variations in the protective value of these sera. In our work this has not been done in all cases, but Table X gives the results of some tests undertaken to determine the neutralizing power of serum from normal horses, and Table XI the results in determining the neutralizing power of the globulin from this normal serum. It will be seen that many among these display a marked neutralizing action when tested against one or more fatal doses of diphtheric toxin. The accepted unit of antitoxin—the amount of antitoxin which will protect the test animal against 100 minimal fatal doses of the diphtheric toxin—represents in reality a fair neutralizing action, for 1 cc. of a serum possessing only a strength of one unit has the power of rendering harmless 100 minimal fatal doses of toxin. Remembering this in considering these tables, it will be seen that, although these sera show only very exceptionally as high a power as one antitoxic unit, yet some of them in amounts of one ccm. protect the test animal (guinea-pig, 250 grm.) against many minimal fatal doses of the toxin—in the case of Horse 137, against 300 minimal fatal doses. Reference to Table XI will also show that the globulins from these sera act in a like manner against the toxins of diphtheria. These sera, however, in many instances do not contain more globulin than others of less neutralizing power, hence the variations in diphtheric antitoxin or neutralizing substances are again demon-

strated to be only one of the factors causing increase in the globulin precipitates, and can but partially explain the variations in these precipitates.

TABLE X.
DETERMINATIONS OF THE NEUTRALIZING POWER OF NORMAL SERA.

Horse.	Weight of Guinea Pig.	Serum + Toxin.			No. of fatal doses protected against.	Result.
126	225	10	cc. +	1.5 f.d.t. ¹⁰		Loss in wt.; no induration. Recovered.
"	315	5	" +	" "		Died in 3 1/2 days.
127	280	5	" +	" "		No loss in wt.; no induration.
"	335	3	" +	" "	0.5	Loss in wt.; induration. Recovered.
"	320	1	" +	" "		Died in 3 1/2 days.
135	365	1/2	" +	" "		No loss in wt.; no induration.
"	270	1/10	" +	" "	15	Induration; loss in weight.
"	248	1/20	" +	" "		Died in 4 1/2 days.
"	243	1/2	" +	5 f.d.t.		?
136	374	"	" +	1.5 "		Much induration.
136	240	1/4	" +	" "		Much induration; loss in weight.
137	255	1/20	" +	1.5 "	30	Gain in weight; no induration.
"	260	1/5	" +	5 f.d.t.	25	No induration; no loss in weight.
137	270	1/10	" +	10 "	100	" " "
"	225	1/100	" +	1.5 "	150	" " "
"	250	1/30	" +	10 "	300	" " "
"	254	1/35	" +	10 "		Died in 3 1/2 days.
139	355	3	" +	1.5 "		Much induration; slight loss in wt.
"	325	1	" +	" "		Died in 48 hours.
140	270	10	" +	" "	0.15	Induration; slight loss in weight.
"	340	5	" +	" "		Died in 3 1/2 days.
141	270	1/4	" +	" "	6	No induration; gain in weight.
"	275	1/10	" +	" "		Loss in wt.; induration.
"	255	1/20	" +	" "		Died in 3 1/2 days.
142	313	10	" +	" "		No induration.
"	365	5	" +	" "	0.3	Induration; no loss in weight.
"	325	3	" +	" "	0.5	Loss in weight; induration.
"	325	1	" +	" "		Died in 3 1/2 days.

All controls of 1 1/2 f.d.t. died in 2 1/2 to 3 1/2 days. Toxic dose corrected for weight in all tests.

It is then not at all surprising that the amount of so-called normal globulin should be a very varying one if we keep in mind all the various factors which must certainly influence its increase and decrease; nor is it curious that no definite amount of increase can be determined for a given increase in antitoxic value, since many of

¹⁰ f. d. t. = minimal fatal dose of toxin.

these same causes are constantly at work masking results. More refined methods of analysis and separation may bring out differences between the substances increased and show us that there is a constant factor, but at present we cannot say more than that the globulins and antitoxin increase and decrease under the same conditions, and respond in the same manner to all tests.

Although the determination of the protective value of sera from normal horses was undertaken by us principally to establish some relation between these values and the amount of globulin precipitate obtained from the sera, still we hoped also that such determinations might in themselves serve as indications of the value of horses for the production of diphtheric antitoxin.

TABLE XI.
DETERMINATIONS OF THE NEUTRALIZING POWER OF THE GLOBULINS
FROM NORMAL SERA.

Horse.	Weight of Guinea Pig.	Globulin sol. ¹¹ and Toxin.	No. of fatal doses protected against.	Result.
135	270	1/2 cc. + 1.5 f.d.t.	3	Gain in weight; no induration.
"	245	1/2 " + 5 "		Died in 7 1/2 days.
137	244	1/5 " + 5 "	25	No induration; no loss in weight.
"	260	1/30 " + 10 "	300	" " " " "

An analysis of the results obtained is so dependent upon one's conception of the relation of the normal protective or neutralizing substances to the true antitoxin, and also upon one's conception of the origin of antitoxins, that some reference to these questions is necessary, although it must be remembered that any discussion of these subjects is at present necessarily largely hypothetical.

It may be generally true that normal horses, the sera of which possess marked neutralizing powers against the diphtheric toxin, are more promising subjects for immunization than are those from whose sera such power is practically absent or only poorly developed; yet this is not the constant experience of ourselves or of others,¹² and

¹¹ Globulin solution contains the same amount of globulin in 1 cc. as was contained in 1 cc. of the serum from which it was derived.

¹² See Bolton, *Journal of Experimental Medicine*, 1896, I, p. 543.

directly opposed to it has been our experience especially with one of our horses. In this case the normal serum showed quite a marked neutralizing action, while, even after long treatment with toxins, only a very weak antitoxic serum was obtained, and the animal, as a source of antitoxin, was abandoned. Such occurrences make us feel that it is possible for the animal body to take care of a poison by other methods than the increased production of anti-substances, for instance by an unusual power of excretion of the poison, or by rendering it inert by destruction rather than by antagonistic action or neutralization. It seems well at least not to allow our conceptions to become too narrow when dealing with these special problems, lest we overlook some side-reactions which may be going on hand in hand with the main process, and may at times even play a major part, and thus account for the behavior of some animals which show neither great systemic disturbances when inoculated, nor produce antitoxins in quantity in response to such inoculations.

Reference to Horse 136 shows on the other hand that an animal possessing only a very weak protective serum primarily, may be perfectly capable of producing very high grade antitoxin. The experience of Meade Bolton confirms our own, for he writes¹³ "that it would seem that the presence or absence of more or less antitoxin normally has no effect upon the ultimate production of artificial antitoxin by inoculation, but its presence enables the inoculation to be made with less risk to the animal." Bolton refers to these substances occurring in apparently normal sera as antitoxin, but we have spoken of them advisedly simply as protective or neutralizing substances, since their identity with the true diphtheric antitoxin is considered by some observers as at least doubtful.

Cobbett¹⁴ has recently published a paper on the question whether the normal horse's serum contains diphtheric antitoxin. To solve the problem of the identity or non-identity of the protective substance with antitoxin he attacks it experimentally, basing his work and conclusions upon an acceptance of the toxin-toxoid composition of diph-

¹³ *Op. cit.*, p. 545.

¹⁴ *Lancet*, 1899, ii, p. 332. Also, *Centralbl. f. Bakt.*, 1899, xxvi, p. 548.

theria-culture filtrates as set forth by Ehrlich. Testing the protective normal sera against the toxins in the manner indicated by Ehrlich, he finds that they act in all respects as typical antitoxins. He concludes that these experiments afford a proof of the identity of these substances. He does not, however, think that it follows that the antitoxin is a normal constituent of the serum of the normal horse, but rather that the diphtheric antitoxin present in the blood of certain men and horses is probably acquired, or, in the case of the young, inherited from the mother, by whom immunity has been acquired. "The presence of antitoxin in these animals cannot therefore," he claims, "be held to throw any light on the origin of antitoxin."

To enter into the question as to whether these protective substances are, in the accepted sense of the term, antitoxins or not, would lead us far afield into a discussion of the mechanism of and bases for the production of immunity in general, and of antitoxins in particular; yet we cannot but feel that, so far, the weight of evidence is on the side of those who hold that these substances are the same in composition as the so-called specific antitoxin, and further, that they are either normally present in the blood or capable of being produced under stress by the cells. By the term "specific" in describing antitoxin we mean derived from a personal infection, or an ancestral infection, near or remote, with the specific organism of diphtheria, or from an inoculation with its poisons.

Considering this subject briefly, we find in some horses, with no individual or ancestral history of infection with the diphtheria bacillus, a substance or substances which will protect test animals from multiple fatal doses of diphtheric poison; we find, also, we believe, both from our own experiments and from those of others, that the normal protective or neutralizing substances are globulins (or associated substances that cannot be differentiated from them) just as are the true antitoxic substances, which are formed during an infection or artificial immunization; and, furthermore, whether we accept or do not accept the views of Ehrlich¹⁵ upon which Cobbett has based

¹⁵ For a consideration of these views, based upon experimental work, see Park and Atkinson, *Journal of Experimental Medicine*, 1898, iii, p. 513.

his conclusions, still the fact remains that his experiments have shown that these protective substances in normal serum act toward the diphtheric toxins in precisely the same manner as do the true antitoxins.

If we look upon these facts as in any way proving the identity of the normal protective substances and specific antitoxin, then a disbelief in the antitoxins and the normal protective substances being normal physiological products or potential products of the cells of antitoxin-producing animals finds for its chief foundation a hypothetical past infection or past or present non-pathogenic association with the germs of diphtheria. Even if such infections or associations could be proven, the problem would simply be referred back in the process of evolution, but in nowise elucidated, since there must have been a point in history where no such infection or association had yet taken place, hence the fundamental question at issue is in regard to the bacterial (toxic) or cellular origin of the antitoxins.

Much weighty and convincing experimental evidence¹⁶ has already been brought forward against such views as those of Fraser¹⁷ "that the antitoxic or immunizing substances originate not from vital reactions upon constituents of the body, but from the toxins themselves, being produced by chemical changes in them, or being actually among their normal ingredients"; so that even assuming the presence of toxins, these would not account for the production of antitoxins, unless the latter were products of the cells, and simply increased in response to toxic action or stimulation.

Since we have, then, as far as we can determine, normal animals producing under normal conditions protective substances, which are, according to our tests, identical with the specific antitoxin produced in animals and man by disease or inoculation; and since, unless we assume in all cases a recent past infection or some constant source of toxin supply, neither of which can in most cases be shown to have a basis in fact, we are forced to turn to the animal economy for the supply of antitoxic substance. Hence we are inclined to look upon these substances as products of certain cells of the bodies of the animals in whose sera they are found, or as products which may be

¹⁶ See Salomonsen and Madsen, *Annales de l'Institut Pasteur*, 1898, xii, p. 763.

¹⁷ *Lancet*, 1898, ii, p. 247.

regarded as potential in the cells of these animals which show no normally present antitoxic bodies, but develop them in the presence of toxins. That this last supposition is not purely hypothetical is indicated, as shown above, by horses with practically no normally present protective bodies, but which develop antitoxic sera in response to inoculations.

That these protective substances subserve some other purpose in the normal physiological economy may or may not be true—but that they must have existed as normal physiological products or potentialities even in the first surviving animal attacked by the specific disease, providing cure resulted from the formation of an antitoxin, seems at least probable, if not undeniable.

It appears to us, therefore, that these substances or antitoxins, have in all likelihood had no absolute dependence upon or relation to toxin even remotely in the history of the race or individual, except as they may have been increased and adapted to the protection of the organism in the presence of an infection;¹⁸ and hence that the normal protective substance and specific diphtheric antitoxin are one and the same substance or substances, which fluctuate within certain limits as do all other physiological products, according to the present need and condition and past history of the animal.

CONCLUSIONS.

The results of the foregoing experiments may be briefly summarized as follows:

The amount of antitoxic substance obtained by precipitation with magnesium sulphate from the blood-serum of the horse corresponds, as nearly as can be determined by the use of test guinea-pigs, in full to the protective power of the serum from which it is obtained, *i. e.* the precipitate from 1 cc. of serum will protect against the same amount of toxin as 1 cc. of the serum itself.

Equal amounts of the precipitates by magnesium sulphate from immunized and non-immunized horses act differently toward toxin; *i. e.* the proportion of protective substance to the precipitate from

¹⁸ Cobbett has recently reported a conclusive observation of the natural occurrence in a horse of nasal and laryngeal diphtheria caused by *Bacillus diphtheriæ* (*Lancet*, Aug. 25, 1900, p. 573).—EDITOR.

non-immunized serum is exceedingly small as compared with the proportion of antitoxin to the precipitate from sera of immunized horses.

The average precipitate from the sera of immunized horses, as obtained by magnesium sulphate, is more abundant than the average precipitate from sera of non-immunized horses.

In the case of the same animal before and after immunization, the serum before immunization gives a less abundant precipitate with magnesium sulphate than the serum tested after immunization.

The proportion of increase per unit of antitoxic strength for the same or different horses is not constant. This may be due to an increase of inactive substances (in their relation to diphtheric toxin) or to imperfect methods of determination.

The precipitates obtained by magnesium sulphate give all the reactions recognized as characteristic of globulins, and as distinguishing them from other albuminous bodies. We are not warranted, then, in the present state of our knowledge, in considering any part of these precipitates as other than globulin. But it does seem warrantable to conclude, from the fact that the globulins of normal serum do not protect, or only in comparatively large amounts, against diphtheric toxin, that new globulins are formed, or rather greatly increased in the serum of immunized horses, and that these globulins protect against the toxin. These increased globulins and the inert globulins (which from obvious causes are a very variable factor) are both precipitated by magnesium sulphate.

Every animal has a physiological and pathological history more or less widely diverging from the normal, hence absolute conformity in the results obtained is not to be expected, at least with our present methods of differentiation.

We desire to express our sincere thanks to Dr. William H. Park, Assistant Director of the Research Laboratory, for the many courtesies shown us throughout the continuance of this work; and also to Dr. George P. Biggs for his valuable assistance in aiding us to procure suitable material for our experiments.

We are especially indebted to Prof. T. Mitchell Prudden of Columbia University for helpful suggestions during the preparation of this article.

THE FRACTIONAL PRECIPITATION OF THE GLOBULIN AND ALBUMIN OF NORMAL HORSE'S SERUM AND DIPHTHERIA ANTITOXIC SERUM, AND THE ANTITOXIC STRENGTH OF THE PRECIPITATES.

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In December, 1899, I published in this Journal¹ a preliminary note on the fractional precipitation of the globulin and albumin of normal horse's serum and diphtheric antitoxic serum. I now present the completed work, though there is still much which I cannot explain and which I have not as yet been able to work out.

The proteids of diphtheria antitoxic serum do not show any determinable differences chemically from those of the normal serum. The globulin precipitate²—which possesses the antitoxic power—increases in quantity but not necessarily proportionately to the antitoxic increase (see the preceding article, p. 47).

The method employed in the precipitation is as follows: 10 cc. of serum are pipetted into a beaker glass of suitable size and diluted up to a volume of about 50 cc. with water; the globulin is then removed by saturating the diluted serum with magnesium sulphate and filtering the precipitated globulin. The globulin precipitate is dissolved in water and again precipitated by magnesium sulphate, filtered off, and washed with saturated magnesium sulphate solution. It is thus freed from the albumin. The filtrates, which contain the albumin, are combined. The globulin derived from the 10 cc. of serum, is dissolved in about 200 cc.

¹ *Journal of Experimental Medicine*, 1899, iv, p. 649.

² The article of Brieger and Boer entitled "Ueber Antitoxine und Toxine" (*Zeitschr. f. Hygiene u. Infectiouskrankh.*, 1896, xxi, p. 259), and the more recent work of Freund and Sternberg, entitled "Ueber Darstellung des Heilkörpers aus dem Diphtherieheilserum" (*ibid.*, 1899, xxxi, p. 429) show that the antitoxin is not carried down mechanically by the globulin, but that there is a true precipitation of the antitoxic substance.

of distilled water and the solution is saturated with sodium chloride. The albuminous filtrate is also saturated with sodium chloride and set aside for the fractional precipitation of the albumin.

The globulin solution saturated with sodium chloride is allowed to stand over night at the room temperature ($15-20^{\circ}\text{C.}$) before filtering off the precipitate which forms upon saturating. A shorter time may do quite as well but I have in all my experiments allowed it to stand over night in order to be sure of saturation. The precipitate after filtering is washed with the saturated sodium chloride solution, dissolved in distilled water and coagulated. 350-400 cc. of water is a convenient volume from which the coagulation of the $R.^{\circ}$ (room temperature) precipitate may be made by bringing the solution to a complete state of boiling and then adding weak acetic acid drop by drop until the liquid is faintly acid. The coagulated $R.^{\circ}$ globulin-precipitate is allowed to settle and is then filtered on a Gooch crucible, washed with hot distilled water, then with absolute alcohol, dried in the air-bath to constant weight, cooled in the desiccator over calcium chloride and weighed.

The beaker containing the filtrate from the $R.^{\circ}$ precipitate is placed in a water-bath, the temperature of which can be regulated easily, and the temperature is raised to 40°C. , sodium chloride having been added to ensure saturation. At this temperature a turbidity makes its appearance and increases until 45°C. is reached. The solution is constantly stirred while the temperature is rising. The $40-45^{\circ}\text{C.}$ precipitate can now be filtered off and the filtrate should run through the filter perfectly clear after 20 or 25 cc. have come through and have been poured back on the filter. If it continues to run through cloudy, the precipitation is not complete and the beaker containing the solution from which the precipitation is being made should be put back in the water-bath and the temperature run up to 45°C. again with constant stirring.

This precipitate also dissolves readily in water, and is coagulated, filtered, washed, dried and weighed as described. In the same manner one obtains a third turbidity at 49°C. and complete precipitation at 54°C. ; a fourth turbidity at 57°C. and complete precipitation at 62°C. These four precipitates are soluble in water. Finally a fifth turbidity appears at 67°C. and there is complete precipitation at 72°C. The final precipitate is not entirely soluble in water but the few insoluble flocks are easily soluble in a little sodium hydroxide, and after neutralization with acetic acid their solution can be added to the general solution of this precipitate. The dissolved precipitates are coagulated, filtered, washed, dried and weighed as described.

TABLE I.

COMPARISON OF THE FRACTIONAL PRECIPITATES OF GLOBULIN OF NORMAL AND ANTITOXIC SERA, SHOWING ALSO THE LOSS OF GLOBULIN IN WEIGHT DURING THE PROCESS OF PRECIPITATION. 10 CC. OF SERUM WERE USED IN EACH CASE.

Temps. of Precip.	R° ppe.	40° ppe.	49° ppe.	57° ppe.	67° ppe.	Sum of Frac.	Total Glob.	Loss of Glob.
Horse No. 137 Normal Serum	.1457	Trace	.0482	.0746	.0071	.2756	.3235	.0479 gms.
137, bled 10/20 '99, 1200 units per cc.	.0648	.1026	.1247	.1899	.3038	.7856	.8292	.0436 gms.
137, bled 10/20 '99, 1200 units per cc.	.4419	.0397	.2970	.0362	Trace	.8144	.8292	.0148 gms.
137, bled 11/9 '99, 650 units per cc.	.1729	.1086	.1253	.0950	.0124	.5142	.5934	.0792 gms.
137, bled 11/14 '99, 600 units per cc.	.0613	.0992	.1007	.1486	.0218	.4316	.5116	.0800 gms.
137, bled 11/18 '99, 400 units per cc.	.2200	.0733	.0842	.0679	Trace	.4454	.4743	.0289 gms.
" " "	.2225	.0724	.1308	.0227	Trace	.4448	.4743	.0295 gms.
" " "	.0853	.0637	.0846	.2038	.0188	.4562	.4743	.0181 gms.
" " "	.1691	.0446	.0713	.1386	Trace	.4236	.4743	.0507 gms.
Horse No. 135, Normal Serum.	.1154	Trace	.0373	.0749	.0399	.2375	.3864	.1489 gms.
135, bled 10/26 '99, 500 units per cc.	.2209	.1408	.1944	.0714	.0149	.6424	.8987	.2563 gms.
Horse No. 136, Normal Serum.	.1005	.0056	.0597	.1450	.0058	.3166	.3536	.0370 gms.
136, bled 10/20 '99, 600 units per cc.	.1277	.0530	.1249	.1919	.1118	.6093	.7389	.1296 gms.
Horse No. 133, Normal Serum.	.0485	.0124	.0378	.0731	.1473	.3191	.3727	.0536 gms.
133, bled 9/18 '99, 400 units per cc.	.1376	.0604	.0995	.1304	.2600	.6879	.7782	.0903 gms.

The final filtrate from the 67-72° C. precipitate fails to give the biuret reaction, and on boiling and subsequent addition of a little acetic acid shows no turbidity. These reactions are common to the globulin of normal and diphtheric antitoxic globulin. Duplicate analyses give frequently different results for corresponding temperatures (see Table I). These differences cannot be accounted for by incomplete precipitation, for if, for any temperature the precipitation was found to be incomplete, the solution was again raised in temperature to the proper degree and the globulin was filtered out and added in its proper place. They could be accounted for by considering each precipitate as caused by the formation of a globulin salt. The amount of the precipitate at any given precipitation tempera-

ture would then depend upon the amount of the compound formed. At the close of the paper I shall consider more fully this hypothesis.

The globulin suffers a loss during the separation (see Table I), and I have found nitrogen in the 67-72° C. filtrates in varying quantities. In order to determine whether the action of sodium chloride had a destructive action on the globulin, three determinations were made by coagulating the globulin from 10 cc. of serum for each experiment.

1. From the watery solution containing some magnesium sulphate which remained with the globulin when it was separated from the albumin.

2. From a one-half saturated sodium chloride solution.

3. From a saturated sodium chloride solution.

Table II gives the results, which show that sodium chloride does not exercise a destructive action on the globulin.³

TABLE II.

COMPARISON OF COAGULATION WEIGHTS OF GLOBULIN, EACH FROM 10 cc. OF SERUM, FROM A MAGNESIUM SULPHATE SOLUTION AND FROM SODIUM CHLORIDE SOLUTIONS.

Horse 133.

Normal serum.

Weak MgSO_4 solution = .3727 grms. of coagulated globulin.

One-half saturated NaCl solution = .3656 grms. of coagulated globulin.

Saturated NaCl solution = .3648 grms. of coagulated globulin.

The globulin fractions from antitoxic serum each contain antitoxin and the final filtrate is free from antitoxin. Some of the antitoxin is always destroyed by the process of precipitation. In this reaction the relation of "diphtheria antitoxin" to globulin is possibly still further shown, the loss in antitoxic power and the loss in weight of the globulin being at the same time and by the same cause.

Table III gives the analyses of a serum containing 400 units per each cubic centimetre which during the process of precipitation lost about 46% of its antitoxic strength.

³Solutions 2 and 3 also contained a little magnesium sulphate which remained with the globulin when it was separated from the albumin.

TABLE III.

THE RELATION OF ANTITOXIC STRENGTH TO WEIGHT OF FRACTIONAL GLOBULIN
PRECIPITATES PER CUBIC CENTIMETRE.

Serum of Horse 133, bled September 18, 1899—400 units per cc.

Temperature of precipitation.	R° ppe.	40° ppe.	49° ppe.	57° ppe.	57° filt.	67° ppe.	Sum of frac.	Original amt of globulin per cc.	Loss of globulin.
Weight of globu- lin precipitates in grms. }	.0457	.0036	.0132	.0078	.0103	.0011	.0714	.0778	.0064
Antitoxic strength of precipitates in units. }	100 to 115	10 to 15	50 to 75	25 to 50	40	10 to 15	195 to 237	Original strength 400 units per cc.	Mean loss of antitoxic strength 184 units = 46%.

This table also shows that antitoxic power is lost during the heating process, while the proteid may be scarcely affected in weight, for if the globulin from the 57° filtrate is substituted for the globulin obtained from this filtrate, *i. e.* the 67° ppe., we have a sum of the fractions .0028 grms. greater in weight than the total globulin. That is, the sum of the fractions and the total globulin are practically the same, and we have a loss of 40% of antitoxic power. We may explain this by considering the antitoxic molecule rearranged by the lower temperature during the process of precipitation without the destruction of the coagulable properties of the proteid.

It will be seen by Table III that there is only a general relationship between the number of antitoxin units and the corresponding weight of globulin.

The destruction of the globulin and antitoxin by the process of precipitation is directly shown by the difference in globulin and antitoxin found in the 57° C. filtrate and the amount found in the precipitate obtained from it.

These reactions of normal and antitoxic globulins toward the salt precipitants, coupled with the fact that normal horse's serum is found to possess decided antitoxic properties against diphtheria toxin and that this antitoxin in normal horse's serum is also found in the globulins, compels us, I believe, to place diphtheria antitoxin among the globulins. The non-correspondence of change in globulin weight to antitoxic increase and decrease, except in a very general way, indi-

icates in immunized as well as in normal serum the presence of other forms of globulin besides the antitoxic, all of which are affected by the group reagents and coagulated by heat in the same way.

The fractional separation of the albumin (Table IV) is carried out as described for the globulin.

The albuminous filtrate which has been saturated with sodium chloride is poured off from the mass of salts⁴ at the bottom of the beaker. The salts are washed with a saturated sodium chloride solution and the washings are added to the main portion of the solution. Some albumin may be held back in the crystals, and in order not to have too large a volume to work with, I have not washed very carefully but have dissolved the crystals and coagulated the proteid which they held. This is afterwards distributed proportionately among the various precipitates. The first precipitate of the albumin makes its appearance at 56° C. and may be filtered off at 61° C. This precipitate is soluble in water. The second precipitate appears at 67° C. and may be filtered off at 72° C. This precipitate is partially soluble, a portion being coagulated and needing weak sodium hydroxide for its solution. The third and fourth precipitates are not soluble, but are coagulated. They are thrown out of solution at 73-76° C. and 77-81° C. respectively. They are at best only slight and in some cases only represented by traces. The final filtrate is free from coagulable proteids and fails to give the biuret reaction. As in the globulin so in the albumin, some of the proteid is lost during the separation.

Dr. Thomas B. Osborne of the Connecticut State Agricultural Station has shown that certain vegetable proteids form definite compounds with

⁴ In the preliminary communication, I stated that a double salt was formed by saturating the magnesium sulphate filtrate, containing the albumin, with sodium chloride. The statement is incorrect. Almost every analysis showed the salt to be sodium sulphate. The following are 3 selected analyses of the salt:

No. 1. 1st crystallization.	
Mg =	9.45%
SO ₄ =	38.26%
H ₂ O =	51.89% determined by
	loss in weight.
	99.60%

No. 3. Recrystallized four times.	
H ₂ O =	56.29%
SO ₄ =	29.81%
Na =	13.9% by difference
	100.00

No. 2. 1st crystallization.	
Mg =	4.54%
No other constituent quantitatively determined. Cl present.	

Impurity of Cl and Mg in 1st crystallization. Cl persisted until fourth crystallization.

mineral acids. Thus, he has separated edestin⁵ mono- and bihydrochlorate. The nitrate, phosphate, sulphate and acetate of edestin are indicated by the reaction. The behavior of the animal globulins and albumins may find their explanation in the same way. The individuals of the globulin group may form sulphates insoluble in saturated magnesium sulphate solution or may form double salts (globulin magnesium sulphate) insoluble in saturated magnesium sulphate solutions. If we saturate the "globulin solution" with sodium chloride at room temperature a precipitate separates out. The precipitate may be accounted for by considering the chlorine or sodium chloride as uniting with or effecting a double decomposition with the globulin sulphate or globulin magnesium sulphate, and the formation of either a simple globulin chloride or double salt of globulin sulphate and chloride, or, perhaps, a complex salt containing globulin magnesium sodium chloride and sulphate. The precipitations at the other temperatures could be accounted for by the same reactions.

TABLE IV.

COMPARISON OF THE FRACTIONAL PRECIPITATES OF ALBUMIN OF NORMAL AND ANTITOXIC SERA; SHOWING ALSO THE LOSS OF ALBUMIN IN WEIGHT DURING THE PROCESS OF PRECIPITATION.

10 cc. of serum were used in each case.

Temperature of precipitation.	56° ppc.	67° ppc.	73° ppc.	78° ppc.	Sum of fractions.	Total albumin.	Loss of albumin.
Horse No. 137, Normal Serum }2259	Traces for 67°, 73° + 78°			.2259	.3127	.0868 grms.
137, bled 10/20 '99, 1200 units per cc. }1558	.0180	Traces for 73° + 78°		.1738	.2046	.0308 grms.
137, bled 11/9 '99, 650 units per cc. }1987	.0204	Traces for 73° + 78°		.2191	.2727	.0536 grms.
137, bled 11/14 '99, 600 units per cc. }1791	.0351	Traces for 73° + 78°		.2142	.2938	.0796 grms.
137, bled 11/18 '99, 400 units per cc. }1253	.0457	73° + 78° = .0080		.1790	.3133	.1343 grms.
Horse No. 135, Normal Serum }0304	.0672	.0074	.0061	.1111	.3680	.2569
135, bled 10/26 '99, 500 units per cc. }1520	.0188	73° + 78° = .0133		.1841	.2341	.0500
Horse No. 136, Normal Serum }2149	.0428	73° + 78° = .0078		.2655	.3618	.0963
136, bled 10/20 '99, 600 units per cc. }0290	.1193	73° + 78° = .0612		.2095	.2715	.0620
Horse No. 133, Normal Serum }2438	.0986	73° + 78° = .0362		.3786	.4146	.0360
133, bled 9/18 '99, 400 units per cc. }0877	.0407	73° + 78° = .0266		.1550	.2101	.0551

⁵ *Jour. of the American Chem. Soc.*, 1899, xxi, No. 6, June.

The precipitation of the albumin by means of sodium chloride could be accounted for in the same manner. At 73° C. and 77° C. we have coagulation and these portions of albumin are therefore beyond this hypothesis.

The presence of the SO_4 is necessary for these fractional precipitations of the globulin and albumin. According to standard text-books some of the globulin can be precipitated from the serum by saturating the serum with sodium chloride at room temperature. I have obtained another precipitate from the room temperature filtrate by saturating with sodium chloride at 56° C. These precipitates are slight however. In a serum containing 600 units of antitoxin per cc. I obtained a precipitate containing only 10 units per cc. by saturating with sodium chloride at room temperature. Halliburton⁶ states that serum albumin is soluble in saturated sodium chloride and magnesium sulphate solutions, yet when the serum has been treated with magnesium sulphate, one can precipitate fractionally the globulin from its watery solution, and the albumin from its magnesium sulphate solution by saturating these solutions with sodium chloride and raising the temperature.

If it be true that we have a series of compounds of globulin and albumin in the described reactions it would account for the differences in the quantities of globulin obtained at corresponding temperatures from duplicate samples, the amount of the precipitate depending upon quantities of the compound formed.

I hope to be able to show later in what form these precipitates of globulin and albumin occur, whether as pure proteids, simple halogen salts, double salts or complex salts.

CONCLUSIONS.

1. The globulins of both normal and diphtheria antitoxic serum exhibit chemically toward reagents the same reactions, being precipitated by magnesium sulphate and split up into fractions in precisely the same way.

2. All of the diphtheric antitoxic power of both normal and immunized serum is always carried by the globulin and its fractional precipitates.

3. During the fractional precipitation of the serum globulin of horses immunized from diphtheria toxin and horses not immunized

⁶ Halliburton, *Text-book of Chemical Physiology and Pathology*, pp. 127 and 236-237.

from diphtheria toxin, some of the globulin is lost, likewise at the same time some of the antitoxic power of the globulin of the immunized serum is lost.⁷

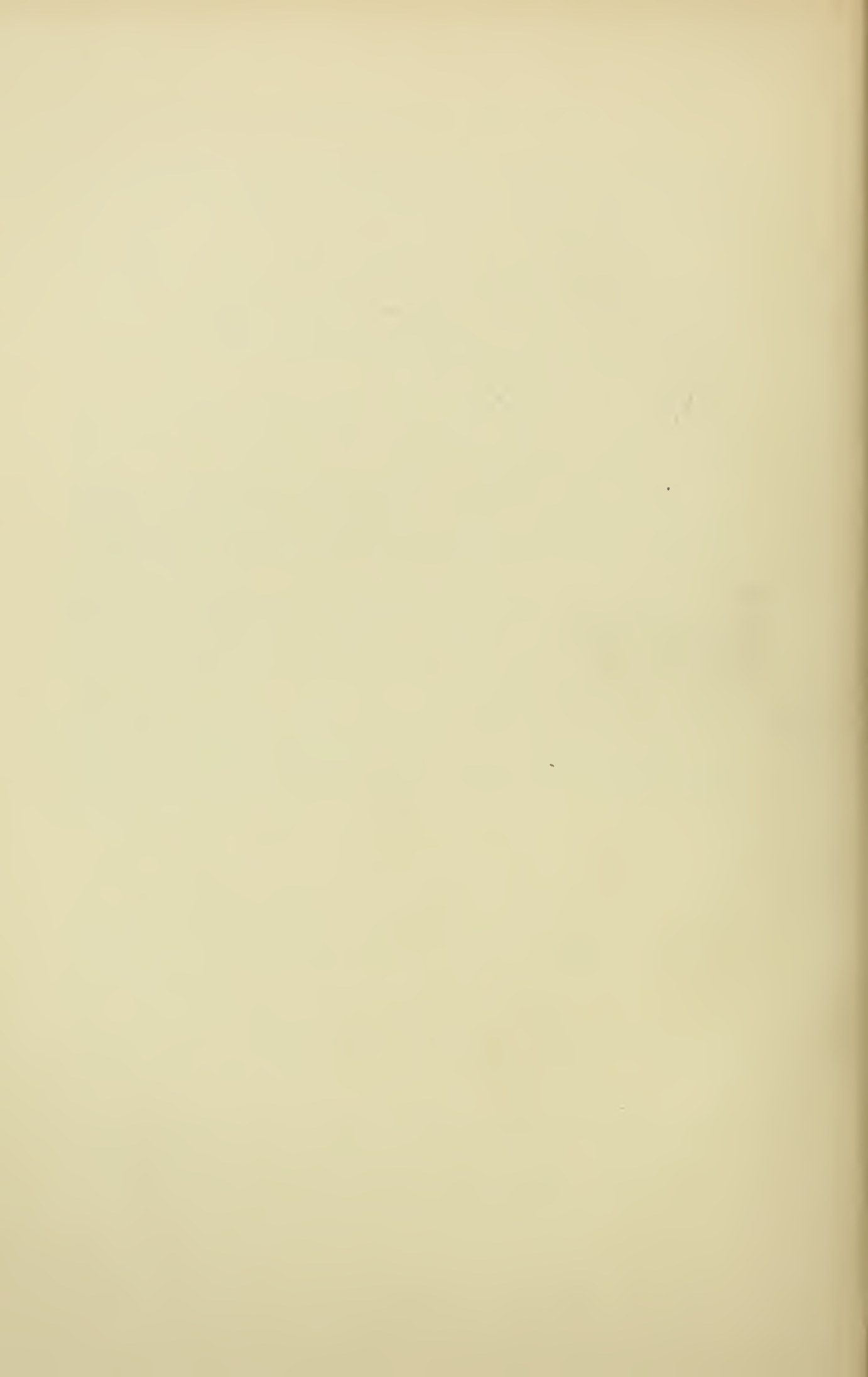
4. These reactions, considered in connection with the fact that different observers as well as we ourselves have found diphtheric antitoxic power in normal horse's serum and that this antitoxin separates with the globulin, strongly incline us to consider "diphtheria antitoxin" a form of globulin.

5. The reactions of globulin, previously separated from the serum by magnesium sulphate, with sodium chloride lead one to think that there is a formation of globulin salts.

6. Since serum albumin in a magnesium sulphate solution gives fractional precipitates at definite temperatures, it seems not improbable that the albumin is precipitated in the form of albumin salts.

I take this opportunity of expressing to Dr. William H. Park, Assistant Director to this laboratory and Dr. Alexander Lambert, Assistant Bacteriologist, my appreciation of their suggestions and kind criticisms during my work.

⁷ On the addition of the fractions of antitoxin tested in guinea pigs, and the addition of the fractions of globulin determined by weighing the coagulated globulin, we find that both have lost, but that the antitoxin has been affected especially by the higher temperature.



REFRACTORY SUBCUTANEOUS ABSCESES CAUSED BY SPOROTHRIX SCHENCKII. A NEW PATHOGENIC FUNGUS.¹

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PLATES II AND III.

In the Bulletin of the Johns Hopkins Hospital for December, 1898, there appeared an article by B. R. Schenck "On Refractory Subcutaneous Abscesses caused by a Fungus possibly related to the *Sporotricha*." "The primary point of infection was on the index finger, whence it extended up the radial side of the arm, following the lymph channels, and giving rise to several circumscribed indurations, which were in part broken down and ulcerated." This infection proved very refractory to treatment. The organism supposed to cause the lesions in this case was obtained in three cultures from two different foci of the disease, twice in pure culture.

Schenck carefully describes the cultural characteristics, the morphology and development and the results of the inoculations of this organism which Dr. Erwin F. Smith, of the United States Department of Agriculture at Washington, tentatively assigns to the genus *Sporotrichum*.

During the last few months we have had occasion to study a case presenting similar refractory subcutaneous abscesses from which an organism has been isolated which is identical in the essential details with the one described by Schenck.

¹ Presented at the Fifteenth Annual Meeting of the Association of American Physicians, held in Washington, May 1-3, 1900. A brief statement concerning the organism was also made by Professor Jordan for the authors at the meeting of the Society of American Bacteriologists in New Haven, Conn., Dec. 28, 1899.

Clinical History (Dr. Perkins):—"On March 16, 1899, Charlie C., aged five years, was brought to my office by his parents, suffering with a sore upon his left index finger, concerning which I elicited the following history: Ten days before he had been using a hammer and had struck himself denuding a surface as large as a split pea on the dorsal surface and over the second joint. This abrasion did not heal up, as his father thought it should, and he applied some 'verdigris salve' to the injury. At present the finger, from the first to the third joints, is swollen to twice its original size, presenting in the centre a deep, well defined, sharp, undermined ulceration, the size of a ten-cent piece. The base of the ulceration is rough and covered with grayish-looking pus. This, when sponged away, leaves a bright red surface; the ulcer extends through the whole thickness of the skin. Surrounding the ulcer over about one-half of the infiltrated area are a large number of vesicles and a few pustules. The dorsal surface of the hand and the extensor surface of the forearm present a chain of swollen lymphatics along which are about twenty nodules the size of a small pea to a large hazel nut. There is no evidence of suppuration in any of them at this time. The little patient does not complain of much pain, either in the finger or arm. The hand is cleaned with green soap and washed with sublimate solution 1 to 2000; the surface of the ulceration mopped over with 95% carbolic acid, powdered boracic acid dusted on, and bichloride gauze and borated cotton applied.

"Subsequently the dressing was changed every second day for ten days and reapplied as at first. At the end of this time ulceration has increased to nearly twice its original size. The nodules are somewhat larger and are getting tender though I cannot detect pus, either by fluctuation or aspiration. On March 28 I began using iodoform as a dressing, but met with no better results. Do what I would, I could not get the finger to improve in the least. The vesicles had formed pustules and the epidermis over nearly all of the infiltrated area had loosened, leaving a raw oozing surface.

"During the first ten days under my care the child seemed to feel reasonably well, but he now began to develop a little fever, and for the next month the temperature ranged up to 100.5° F. There developed about April 1 an annoying and persistent cough and coryza. Early in April the swollen lymphatics began to suppurate. I opened and curetted eleven abscesses in the next week. Some contained not more than thirty minims, others probably four drams of pus. Upon opening one large abscess about the middle of the forearm, I was surprised to

see it drain another smaller one four inches from the incision. This was refilled with the irrigating solution and opened. The fistulous tract was irrigated and a swab with carbolic acid upon it was passed through. The pus from the abscesses was of a mahogany color, thick and tenacious.

"Up to this time (May 25, 1899), I have opened twenty-one abscesses, four have opened spontaneously and still there are more to follow.

"The lymphatic glands in the axilla and neck on the left side have been inflamed, but are smaller now and bid fair to return to their normal size without suppurating. The abscesses which have been opened have shown considerable destruction of the fascia, intermuscular septa and skin.

"Suspecting farcy and with the expectation of having my opinion confirmed, I inoculated two tubes with a quantity of pus from an uncontaminated abscess and mailed them May 2 to Prof. L. Hektoen for examination. At his request, I again sent him tubes about May 9 or 10.

"The family being poor, I have had the mother dress the arm part the time. I once saw her doing this when she had a cut over one-half inch long on the thumb. The quantity of pus from the sores was large at this particular time and it seems impossible that she did not get germs into the wound. This fact and that the patient has mingled with several other children in the family have convinced me that the danger of infection is not very great.

"Internal treatment has varied somewhat: Cough mixtures for two or three weeks; calcium sulphide in $\frac{1}{4}$ grain doses; corrosive sublimate 1/100 grain with quinine sulphate 1 grain. The last named associated with wet dressings of 1 to 100 carbolic acid seemed to do the most good. Two small photographs were taken before any of the abscesses had been opened. A larger one was made May 25, 1899, which shows the present condition very well (Plate II, Fig. 1). The boy's condition has improved very materially and my opinion is that he will fully recover in another month" (which proved to be the case).

BACTERIOLOGICAL EXAMINATION.—The glycerine-agar tubes, inoculated by Dr. Perkins on May 2 and again on May 10, all developed pure cultures of the organism in question. After about 6 days, the last four being in the incubator at 37° C., there appeared several greyish-white, raised, irregular colonies, about as large as a pin's head, confined exclusively to the area of the surface of the medium covered by the bloody exudate. In one tube similar but smaller growths

appeared in a small quantity of exudate accidentally smeared upon the glass. The colonies were rather dense and viscid; considerable increase took place and wrinkled masses formed. Repeated subcultures upon various media show the following cultural characteristics:

Agar (Plate II, Figs. 2-4).—At the end of 24 hours in the incubator there is some growth of a greyish color and granular in appearance along the streak on plain and glycerine agar. After 48 hours the growth appears as a delicate, slightly raised, whitish line with symmetrical feathery fringes and some hairy downgrowth into the substance of the agar. At the end of 72 hours the growth assumes the form of a band with numerous transverse wrinkles; in a couple of days more the surface becomes more markedly corrugated and looks like the chains of mountains on a map. About the 7th day the growth, which has increased some in thickness, becomes light brownish in color, the margins being smooth and wavy, marked by shallow transverse grooves. Still later the growth becomes distinctly and even dark brown, the surface wrinkled and velvety, in some cases covered by a very delicate fuzz. The medium becomes slightly brownish.

On wort-agar a thick yellowish, wrinkled membrane develops, extending some distance upon the sides of the tube. This, or glucose-agar, is one of the best media for rapid growth.

The individual colonies upon glycerine-agar plates appear at the end of 2 to 4 days as irregular, greyish-white dots, 0.4-1 mm. in diameter, resembling somewhat minute flakes of snow. Under the microscope they are made of a central network of threads which at the periphery grow outward in a radiating manner and become tipped with small clusters of minute dots; similar dots (conidia) also appear along the sides of the outgrowing threads (Plate III, Fig. 5).

Individual colonies on agar slants, after three or four days, appear as minute shreds which gradually develop into round, circumscribed, raised, somewhat pearly masses which send fluffy prolongations into the medium; later the surface of the spreading colony becomes raised into irregular wrinkles and eventually a brownish color generally appears.

The early surface-growth in the glucose agar stab presents a whitish, heaped up centre and delicate radiating margins; with time a uniform growth develops along the stab with fine, thickly set, lateral branches. No fermentation.

In one or two instances streak cultures upon agar have resulted in a flat, granular, sparse growth only, consisting almost wholly of round and

oblong budding bodies. Subcultures from such growth give rise to characteristic folded and wrinkled membranes.

Blood serum.—In 48 hours small colonies appear which are covered by a white frosting; the growth increases slowly but presents no special characteristics. There is no liquefaction.

Gelatin.—The deep growth is confined to the upper end of the stab; it increases slowly and sends out lateral branches which are longest immediately underneath the surface which becomes covered by a flat, spreading layer. At the end of about 6 to 7 days a slight liquefaction is apparent; in about 14 to 18 days later the liquefaction is nearly complete, the surface is covered by a dense membrane which has a tendency to sink down into the clear liquid.

Wort-gelatin, acid gelatin and 4% glycerine gelatin are more favorable for growth, and liquefaction takes place earlier; on shaking, the thick surface layer falls to the bottom, afterwards a new layer forms on the surface and delicate threads may be seen growing vertically along the space of the tube. The liquid gelatin remains clear.

Potato.—Twenty-four-hour cultures show a slight brownish-grey or yellowish nodular growth which increases quite rapidly, becomes raised and wrinkled, the surface presenting smaller and larger districts of a white, frosted appearance. The older growths become discolored at the same time as the potato is darkened.

Milk.—Litmus milk is not changed in color and not coagulated; but slight growth takes place.

Bouillon.—The growth is fairly abundant; little tufts or shreddy masses form which settle at the bottom or cling to the sides of the tube; a white, crumbling surface-film is occasionally observed; no change in reaction. The rate of growth seems about equal in plain, glucose, and glycerine-bouillon.

Vegetable infusions.—The fungus grows in hay, turnip, carrot and potato infusions as a whitish, flocculent precipitate, the fluid remaining clear.

Hydrant water.—A very slight growth?

Gasparini's Starch.—A flat, slightly raised, greyish-white, hard layer forms rather slowly.

Fermentation Test.—Glucose, lactose and saccharose bouillon, prepared according to the method of Theobald Smith,² shows a characteristic growth in the aërobic bulb, the anaërobic tube is unchanged and there is no gas formed.

² *Journal of Experimental Medicine*, 1897, ii, p. 546.

Anaërobiosis.—There is no growth in the tubes in Buchner's jars.

Temperature.—The optimum temperature for development would seem to be about 37° C.; good growth occurs at the room temperature but at a much slower rate. No growth occurs at a temperature just above the freezing point, but cultures kept at this temperature for 5 weeks remain alive and give rise to vigorous new growths on reinoculation.

Thermal Death-Point.—The organism is killed by an exposure to 60° C. for $4\frac{1}{2}$ minutes; a slight growth occurred after an exposure to this temperature for 4 minutes. No growth could be obtained after exposure to 59° for 10 or 5 minutes, to 61° and 62° for 2 minutes, to 65° for $1\frac{1}{2}$ minutes.

MORPHOLOGY.—Cover-slips from the cultures, stained with methylene blue, show masses of more or less parallel, or tangled, straight or curved, unevenly stained, rather thick threads with rather infrequent true side-branches. Interspersed among the threads lie numbers of ovate or apiculate bodies, from 3 to 5μ in their longest diameter. In the stained preparations no definite connection is to be made out between the majority of the bodies and the threads. In some preparations the bodies predominate. Occasionally bodies are seen connected with a thread by a small pedicle. The threads seem thinner when stained with Gram's method which gives them a light violet, granular appearance with irregular clear spaces. Gram's method stains the spore-like bodies a deep blue; occasionally there is an unstained area in their interior. Some spores appear to be growing out to form short threads.

In the unstained preparations and in hanging drop cultures of bouillon and gelatin, the threads of the mycelium are seen to be doubly contoured; the protoplasm is somewhat granular and interrupted at fairly regular intervals by transverse septa; the diameter of the threads varies somewhat, the average being about 2μ ; the branches are not frequent and do not bear any fixed relations to the septa.

In the hanging drop cultures the relations of the conidia to the mycelium are very nicely shown. The spore-bearing branches, which grow out in a radiating manner from the central felt-work, are commonly tipped by a cluster of from three to six or more conidia which, in the case of the larger clusters, are attached by the smaller end to the slightly expanded extremity of the branch. Similar ovate buds also arise from the sides of the hyphæ at shorter or longer intervals.

The spores are also doubly contoured and granular, resembling very much yeast cells.

These various features are well shown in the photographs of the growing hanging-drop cultures (Plate III, Figs. 6 and 7).

The attachment, by means of short pedicles, of the spores to the threads is very easily severed as shown by the difficulty in obtaining stained preparations with the spores in situ.

Development.—When placed in the hanging drop the conidia grow out into one or more straight germ tubes which spring from either, or both ends, or from the sides. These embryonal threads again give rise to lateral or terminal buds, which in all particulars resemble the spores and some of which form branching spore-producing threads, so that in the early stages very peculiar looking bodies are produced.

HISTOLOGICAL EXAMINATION.—A small bit of skin including part of the wall of an abscess was excised by Dr. Perkins and sent to me in alcohol. The sections show some thickening of the epithelial layer; there are rather broad interpapillary downgrowths. On the cutaneous surface there is much horny material, altered red blood-globules, detritus and polymorphonuclear leukocytes often collected into dense foci within and upon the horny layer. The epithelium and the cutis show many leukocytes, with long drawn-out thread-like and contorted nuclei, apparently going in various directions and also aggregated into small groups. The vessels of the cutis are congested.

Along one side and part of the lower or deep surface of the section there is considerable bloody and leukocytic exudate; the adjacent tissue is infiltrated with leukocytes with greatly elongated nuclei which might be taken for fragments of fungus threads.

In sections stained by the Weigert and Gram methods are short threads which cannot be otherwise distinguished from the leukocytic nuclei mentioned. With this possible exception no micro-organisms are seen.

ANIMAL EXPERIMENTS.—Rabbits.—I-II. Injections of 2 cc. of a 24-hour bouillon culture into the abdominal cavity and into the ear vein of two large rabbits respectively produced no symptoms in 8 weeks.

III. A few drops of a bouillon culture inserted into the anterior chambers of the eyes of a small rabbit in 3 days produced a moderate whitish exudate, and a few, pin-head sized, white clumps formed which persisted for 2 days after which rapid recovery took place.

Guinea-pigs.—I-III. Intraperitoneal injections of 2 cc. of a suspension in bouillon did not produce any symptoms or changes after 6 weeks in the case of three animals.

IV. May 17 I injected a guinea-pig with 2 cc. into the subcutaneous

tissue over the abdomen. A number of small, pea-sized, firm nodules developed about the site of the injection. The animal died May 29. The subcutaneous nodules were small abscesses; in the pus were numerous oval and oblong bodies staining irregularly with methylene-blue and with Gram's method. No other organisms were found and pure growths of the sporothrix developed in the cultures from the pus. The internal organs were sterile and of normal histological structure.

V. A guinea-pig inoculated subcutaneously with 2 cc. July 25 died Aug. 18. There were no local changes visible and cultures from the internal organs remained sterile. At this time a number of guinea-pigs in the laboratory were dying from unknown causes.

Dogs.—I. Intravenous injection of 1 cc. of a suspension in bouillon did not produce any symptoms; all organs appeared to be normal and proved to be sterile when the animal was killed on the 34th day.

II. Two cc. of a bouillon suspension was injected into the subcutaneous tissue of a large, female dog. In a few days a small swelling appeared which was quite tender; a diffuse slightly tender induration remained for some time.

Twenty-eight days after the first injection 2 cc. were again inoculated under the skin of the thigh followed by the development of a small, fluctuating swelling. There was no enlargement of the corresponding inguinal glands. The animal was killed on the 15th day. The internal organs were normal and sterile. At the site of the first injection was an area of scar tissue which on microscopic examination showed islands of marked round cell infiltration; no organisms could be demonstrated in the sections and the cultures from the scar tissue remained sterile.

At the site of the second injection was a small, soft, whitish spot or cavity containing a gelatinous material, smears and cultures from which showed the organisms injected to be present in fair numbers. The sections, stained by Gram's method, showed organisms in fair numbers among the leukocytes in the little cavity which was enclosed in a recent fibrous tissue. The organisms resembled somewhat the conidia of the fungus, stained irregularly, and varied in size, being oval or oblong and from 2 to 3 or 4 μ in length.

III. Intraperitoneal injection of 2 cc. of a bouillon suspension did not produce any lesions, the organs being healthy and sterile, when the animal was killed on the 26th day.

White Rats.—I. Subcutaneous injection of 2 cc. of a bouillon culture did not seem to have any effect, the animal remaining well and fat.

II. About two months after the intraperitoneal injection of 2 cc. of

a bouillon culture, during which time the animal remained well, it was noticed that the scrotum was very large and dragged on the ground, interfering with the animal's movements; the hind legs were oedematous. Killed by chloroform. The larger part of the small intestine and omentum occupied the two sides of the scrotum. The peritoneum over the lower third of the abdominal cavity and lining the scrotal cavities, was covered with numerous yellowish, tuberculiform nodules, arranged now singly, now in groups or clusters. On incision each nodule was found to contain a quantity of yellowish-grey, viscid purulent material composed of leukocytes and innumerable, irregularly stained (Gram), oblong and oval conidia from 2 to 4 μ in length. Many presented a transversely striated appearance, in others there was a clear spot near one end. There were no threads present in the pus. The other organs seemed normal; cultures from them and from the heart's blood remained sterile; no organisms were present in any of the smears. The cultures from the abdominal nodules gave rise to innumerable characteristic colonies solely of the organisms injected.

The sections from the peritoneal nodules show small cavities enclosed in recent fibrous tissue; these cavities contain polymorphonuclear leukocytes, nuclear detritus, and the conidia of the organism in large numbers (Plate III, Fig. 8), most numerous just around the inside of the wall; here they are scattered about singly or arranged in groups; nearly all are extracellular but intracellular groups occur; if the Gram stained sections be allowed to remain in alcohol for a little longer time than that just sufficient for decolorization then the organisms lose their stain largely and may appear as cocci or short, thick bacilli of various sizes. The organisms cannot be distinguished in the hematoxylin and eosin specimens. Giant cells are not seen in the interior of the abscesses but in the recent fibrous tissue of the walls lies an occasional multinuclear cell of the tubercular type. In a few places the appearances indicate beginning abscesses; the first effect of the organism is a necrosis of the tissue followed by cell accumulation; it cannot be made out clearly just how the organisms are carried, or go, from an older to a recent focus; the presence of bodies within cells, especially leukocytes, indicates that they may be transported by wandering cells; a few single bodies are found scattered about in the tissue outside of the abscesses.

Grey mice.—I. Intraperitoneal injection of .5 cc. of a bouillon suspension produced death in 2 days. There was a purulent peritonitis, the organism being recovered from the exudate in pure growth. The internal organs were sterile and normal.

II. Subcutaneous injection of .5 cc. was followed by death after 2 days. There were no special changes and the cultures remained sterile.

III. Subcutaneous injection of .5 cc. was followed by evident symptoms of illness ending in apparent recovery. The mouse died 18 days afterwards, but on account of beginning decomposition no cultures were made.

IV-V-VI. Subcutaneous injection of .5 cc. Twenty days later one died, but the body was eaten by one of the survivors. This died two days later; there was some induration about the site of the inoculation, the tissues being dry and shrunken. The cultures and smear preparations were negative. About this time the third mouse showed considerable shrinking about the injection—at the root of the tail; a small, dry ulcer formed, one of the extremities appeared fixed and rigid so that the animal limped a good deal. It became thin and died four weeks after the inoculation. The tissues about the ulcer were firm and fibrous; underneath the skin over the upper part of the right thigh and extending upon the under surface of the abdomen was a collection of whitish, viscid, caseous pus which contained oval and oblong bodies in large numbers, and growing readily on glucose agar. The internal organs appeared normal both on gross and microscopic examination and the smears from them did not show any organisms.

White and tame mice.—Four white mice were inoculated with .5 cc. of a 48-hour old bouillon culture under the skin at the root of the tail. All reacted in about the same way. None died soon after the injection. In the 2nd or 3rd week they appeared to be getting thin. Beginning at the root of the tail and extending for a variable distance over the back there now formed a hairless, red area with an uneven surface; scattered over it were occasional yellow spots. The posterior part of the body seemed more or less shrunken, the hind legs stiff, one often more so than the other; in one mouse the left posterior extremity seemed fixed and drawn up against the body. I killed one with chloroform on the 23rd day, one died spontaneously on the 26th, one on the 35th, and the fourth on the 40th day. In all there was found some undermining of the edges of the ulcerated surfaces described; the ulcer was superficial and did not extend into the muscles; at some points the tissues about the margins were firm and fibrous; where the skin was undermined small quantities of semisolid cheesy material were found; sometimes such collections would extend for a considerable distance in the subcutaneous tissue; this material was composed largely of leukocytes and among them were found, in varying numbers, organisms like the conidia of the

fungus; in one animal there were numerous cocci also; in this case the cultures were mixed, most of the colonies being cocci, but quite a few colonies of the fungus developed. In all cases the internal organs were normal with the exception of a possible enlargement of the abdominal lymph glands; no organisms were found in the smears and the cultures remained sterile from the organs, which, including the lymph glands, were fixed in Zenker's fluid; the sections showed no changes and organisms were not found in those stained by Gram's method or otherwise.

In order to study the evolution of the anatomical changes produced by this organism, four tame mice were injected simultaneously into the abdomen with 1 cc. of a bouillon suspension of the same culture and killed at successive intervals of one week each. At the end of the first week a number of small nodules the size of a pin's head had developed near the site of the injection and in the omentum near the spleen which was adherent to the stomach; the retroperitoneal glands were not enlarged; the cultures from the nodules remained sterile; the sections (Weigert, hæmatoxylin, etc.) of the nodules show accumulations of cells with marked nuclear fragmentation and the formation of fibrin especially at the periphery; scattered about are oval and lanceolate organisms often in groups of 4-6 or more.

At the end of the second week there were found nodules, a little larger, scattered principally over the peritoneum of the serotal pouches. Cultures successful. Sections show the nodules to consist largely of a cellular granulation-tissue with smaller foci of marked nuclear disintegration containing a much larger number of organisms than the nodules of a week's duration.

The third mouse died spontaneously on the 20th day after the injection. There was a small ulcer over the lower part of the abdomen, and innumerable yellowish areas and more diffuse flat thickenings with distinct spots of viscid softening over all parts of the peritoneum, the under surface of the liver, etc. Cultures successful. The sections show the tissue to be more fibrous while the foci of softening contain granular and amorphous detritus, small nuclear clumps and innumerable organisms often aggregated into heaps which appear as blue spots visible to the naked eye. In none of these nor in any of the animals were fungous threads found in the lesions.

The fourth died spontaneously and was eaten in the abdomen by another mouse in the same cage.

There can therefore be no question but that the spore-forms multiply in the lesions they produce in susceptible animals.

Pigeons.—Subcutaneous injections in two white pigeons of 2 cc. of bouillon cultures did not produce any lesions or symptoms in 3 weeks.

There can be no doubt in regard to the identity of the organism described by Schenck and the one described in this article. The two correspond morphologically and culturally and their pathogenic actions in animals are the same with the exception of a few easily explainable, insignificant differences. Schenck observed a general infection after subcutaneous injections in mice; as far as I know this did not occur in any of the animals that I used. And I obtained more decided pathogenic effects in guinea-pigs than Schenck seems to have done. These are differences that might reside just as well in the different races of animals used as in the organisms. In a personal communication Schenck states that on comparison the two organisms appear identical in form and in culture. Through the kindness of Dr. Schenck I was enabled to compare the two organisms and I could not detect any distinguishing differences either culturally or morphologically. The injection of Schenck's fungus into the abdominal cavity of a mouse (December 5, 1899), was followed by death after four weeks, and the anatomical and histological characteristics of the lesions did not differ in any essential from those of the lesions produced by the organism from Dr. Perkins' case.

It is regrettable that circumstances prevented the demonstration of the organism in the human lesions. More thorough study of the histological changes produced by the organism in man are also desirable. An abscess should be excised in an early stage of formation.

It is exceedingly interesting to note that we have here a pathogenic fungus that in the lesions it produces in animals exists in the spore-form, or in a modified spore-form, and that it undoubtedly multiplies as such; threads do not seem to develop in the tissues of susceptible animals. The exact manner in which the spores reproduce themselves under these circumstances I have not attempted to determine.

The fungus produces a slow, circumscribed and nodular inflammation with necrosis and pus formation in the centre and the development of granulation and fibrous tissue at the periphery—encapsulation. This is seen especially well in the abdominal cavity of white

rats and mice. The destruction of tissue after subcutaneous inoculation in mice may be quite extensive, large ulcerated surfaces with undermining and purulent infiltrations at the margins being the result. In less susceptible animals, such as the dog, dense areas of scar tissue are produced.

A characteristic clinical feature of the human cases is the refractory nature of the subcutaneous abscesses. This was pronounced in both Schenck's case and in the case under Dr. Perkins' care. Brayton³ describes a similar case clinically; it occurred in a healthy, young florist who punctured a finger with a wire while making bouquets; a succession of chronic abscesses with gelatinous contents appeared, extending during a period of two months from the finger to the elbow, much scarring being left behind. This case was not examined bacteriologically.

The three cases have much in common; in all a succession of similar, refractory, small abscesses of the upper extremity developed consequent upon injury by similar means; in Schenck's case the scratch of the skin of the finger by a nail; in Perkins' case the blow upon the finger by a hammer; and in Brayton's case the puncture of a finger by a wire.

DESCRIPTION OF PLATES II AND III.

PLATE II.

Fig. 1.—Photograph of arm of patient, showing ulcers and sears, at a late stage of the lesions.

Fig. 2.—An original culture of the sporothrix, three weeks old.

Fig. 3.—Slant culture on glucose agar, 4 days old.

Fig. 4.—Slant culture on glucose agar, 8 days old.

PLATE III.

Fig. 5.—Colonies on glycerine-agar plate, 48 hours old.

Fig. 6.—Margin of living hanging drop culture (gelatine). $\times 150$.

Fig. 7.—Same as Fig. 6. $\times 1000$. Unstained living culture.

Fig. 8.—Photograph of section of abdominal nodule in white rat. $\times 1000$. Gram's stain. Cells and spores, the latter oblong and deeply colored.

³ *Indianapolis Medical Journal*, 1899, xviii, p. 272.

90
1



FIG. 1.



FIG. 4.



FIG. 3.



FIG. 2.

90²

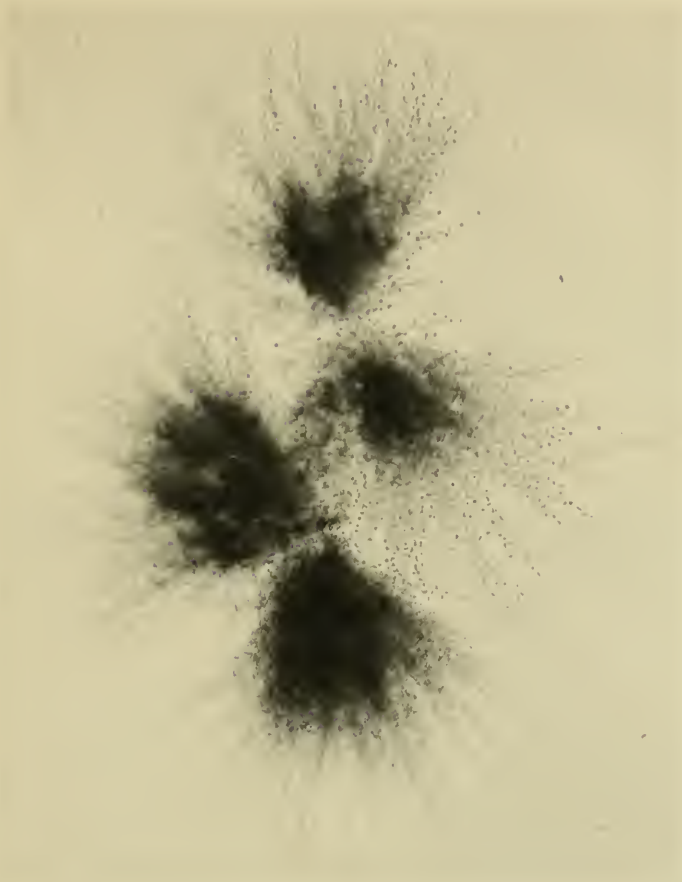


FIG. 5.



FIG. 7.

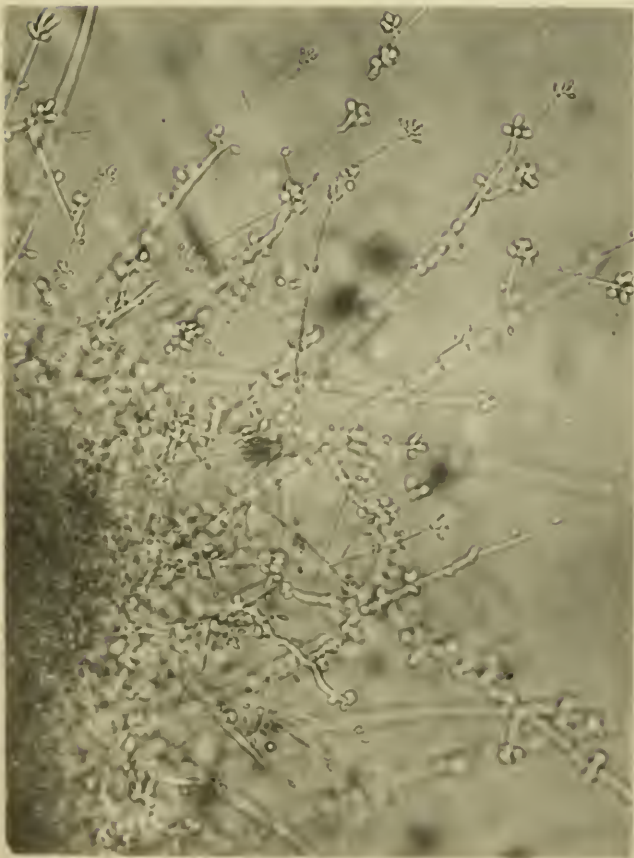


FIG. 6.

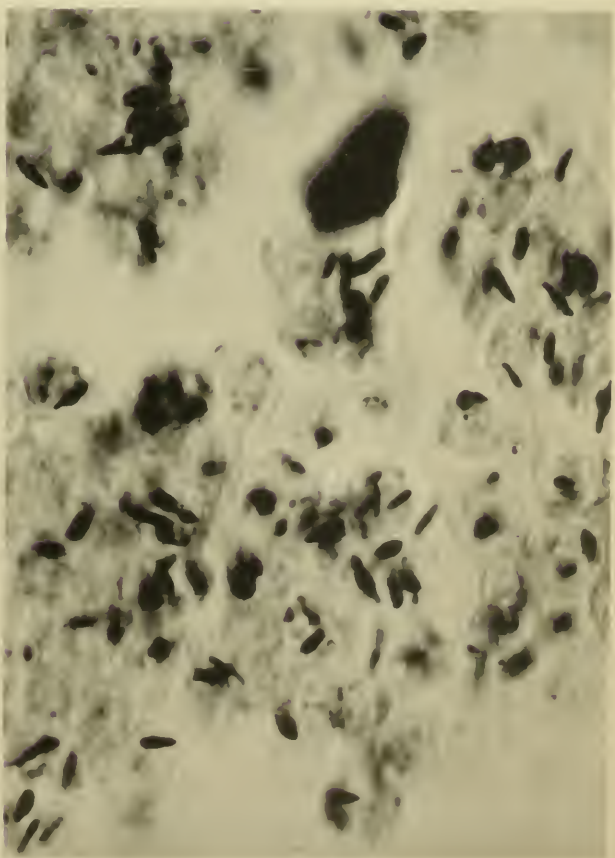


FIG. 8.

PATHOLOGICAL REPORT ON A CASE OF DERMATITIS
VESICULO-BULLOSA ET GANGRÆNOSA MUTILANS
MANUUM (DUHRING), WITH A CONSIDERATION OF
THE RELATIONS OF VASCULAR AND NERVOUS
CHANGES TO SPONTANEOUS GANGRENE AND RAY-
NAUD'S DISEASE.¹

BY WILLIAM G. SPILLER, M. D.

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PLATES IV AND V.

This case was reported clinically by Dr. L. A. Duhring² in 1892 under the title of a "Case of Dermatitis Vesiculosa Neurotraumatica of Forearm." Dr. Duhring thought that the case was obscure and difficult to classify, and notwithstanding the presence of hysteria he believed that the symptoms could possibly be explained by regarding the process as a traumatic ascending multiple neuritis, although he was guarded in expressing this view.

A later clinical history was published by Dr. Sinkler³ in 1897, in which much of the earlier history given by Dr. Duhring is included. A brief abstract of Dr. Sinkler's paper is as follows:

The woman, A. A., was 35 years of age in 1897. For years she had had many symptoms of general nervous disorder which included frequent and protracted attacks of gagging and vomiting, palpitation of the heart, crying spells and globus hystericus. In Sept., 1890, she was burnt with a flat-iron on the flexor surface of the left forearm just above the wrist, the area being about the size of a silver dollar. The burn was superficial but did not heal readily or completely, and from some unknown cause began to break out anew. Within a month of the acci-

¹ Read before the Section on Neurology and Medical Jurisprudence of the American Medical Association, June 5, 1900.

² *International Medical Magazine*, 1892, i, p. 140.

³ *Journal of Nervous and Mental Disease*, 1897, xxiv, p. 687.

dent it began to show a superficial gangrenous patch which remained about six weeks. The whole forearm became reddened and the seat of throbbing and darting pain. About six weeks after the accident the burn seemed to be nearly healed, and then a single pimple, a papulo-vesicle, formed on the extensor surface of the forearm near the burn. In a week or two this lesion ulcerated and crusted, and then other similar pimples formed near the original one; some of them vesicles and some blebs, covering by degrees the greater portion of the wrist. The morbid process continued on the left wrist and upper part of the hand for three years, migrating from place to place and breaking out anew as soon as any point became healed. By the early part of 1894 the left arm was entirely healed.

Just at the time of the healing of the left arm a papulo-vesicle similar to those which had invaded the *left* arm appeared at the end of the *right* index finger. This followed the same course of breaking out into an ulcer and then healing; new papulo-vesicles formed on this finger and then attacked the adjoining fingers. The affection subsequently spread to the dorsal and palmar surfaces of the hand and finally implicated all of the fingers. At times gangrenous patches appeared on the fingers, followed by sloughing and more or less loss of tissue. In this way the fingers and thumb were lost. The patient suffered from pain in the right hand. Objective sensation was unimpaired. The sloughing began with discoloration of the skin and the skin rapidly became black and dry and the slough was thrown off leaving a granulating surface. The urine was free from sugar.

Dr. Sinkler concluded after a careful study of the case that the disease was a trophoneurosis dependent upon an hysterical diathesis.

I made a brief examination of the patient in June, 1899, and observed certain symptoms that caused me to believe that the woman had Graves' disease. I made no notes of her condition, knowing that the case had been studied clinically by Dr. Duhring and Dr. Sinkler. Dr. Sinkler later informed me that the patient had marked evidences of Graves' disease; at least she had distinct exophthalmos and rapid heart's action without much, if any, thyroid enlargement.

In June, 1899, the right hand was amputated by Dr. W. J. Taylor just above the wrist and was given to me for examination (see Plate IV, Fig. 1). The part had become useless and pus had formed in the stump of the fingers. Dr. Sinkler tells me that the patient had

been for years an opium eater. After the amputation she did very well and was improving steadily when one day she received a large quantity of opium pills from some friends. She probably took several of these, as in the evening after the visit of her friends she vomited and was found in a condition of stupor. The next morning she had a convulsion. The urine was examined but no evidence of nephritis was obtained. The convulsions became more frequent, the stupor continued, and she died on the third or fourth day after taking the opium.

Previous to her death Dr. Duhring⁴ published another clinical report of this patient and a beautiful colored drawing of the right hand.

Dr. C. W. Burr made the necropsy and kindly placed at my disposal the brain, cord and some of the peripheral nerves.

Dr. Joseph Walsh made a bacteriological examination of the amputated hand. Important results were not expected from this examination as the parts were exposed to various kinds of infection. His report is: "Aërobic and anaërobic cultures were made from suppurative foci on the fingers and hands and from non-suppurative portions. The results were positive and pure cultures of staphylococci were obtained, the great majority being white staphylococci. Inoculation into guinea-pigs of pus from the suppurating foci and of the pure cultures in agar produced no effect."

The microscopical examination of the tissue was made by me.

Right upper extremity: Ulnar nerve and artery.—The ulnar artery taken from the amputated limb is much thickened, both in the media and intima, especially in the latter. The lumen at one part is almost entirely filled by an organized thrombus (see Plate IV, Fig. 2). An elastic membrane is seen along one side of this thrombus. This thrombus is separated from the intima by a clear space throughout most of its extent.

The nerve fibres of the ulnar nerve in the same section in which the thrombosed ulnar artery is found, appear to be normal by ammonia carmine and the Weigert hæmatoxylin stains. The ulnar artery towards its peripheral terminations is still much thickened, and yet the nerve fibres of the accompanying nerve are normal by ammonia carmine or

⁴ *International Atlas of Rare Skin Diseases*, xiv, March 22, 1899.

Weigert's hamatoxylin stain, or at most are very slightly altered. A section of the ulnar nerve taken from the amputated limb and stained by Marchi's method shows only a moderate amount of black masses within the nerve fibres, and it is questionable whether the nerve could be regarded as degenerated from the evidence furnished by this method.

Nerve fibres from the right hand—I cannot say which nerve—taken just above the finger and stained in the fresh state by osmic acid and teased appear to be normal. The same is true of nerve fibres taken from the lower third of the right forearm.

Median nerve.—A piece of the median nerve, taken about 8 cm. above its termination in the stump, shows some proliferation of the endoneurium with possibly an atrophy of some of the nerve fibres. The extreme end of the branch to the middle finger is considerably degenerated and the endoneurium is much proliferated. Many nerve fibres have disappeared and the small accompanying arteries are much thickened. The extreme end of the branch to the index finger shows some degeneration of the nerve bundles. The extreme end of the branch to the thumb shows distinct degeneration of nerve fibres and proliferation of the endoneurium, but many nerve fibres are still present.

A section from the median nerve of the amputated hand and forearm stained by the Marchi method shows degeneration in some of the nerve fibres, but the degeneration is not excessive and is not so great as that seen in the left brachial plexus. Masses stained black by the osmic acid are seen in many of the fibres.

Radial nerve.—The radial nerve from the amputated part, taken 6 cm. above the styloid process of the radius, shows distinct diminution in the number of nerve fibres and overgrowth of the endoneurium (see Plate V, Fig. 3), but the degeneration is slight in comparison with that of the distal portion of the branch to the thumb. With the Marchi stain the degeneration 6 cm. above the styloid process is considerable. At the distal portion of the branch of the radial nerve to the thumb (Plate V, Fig. 4) the alteration of the nerve fibres is greater than in any other nerve. The fibres here have mostly disappeared and the connective tissue is greatly proliferated.

A branch of the radial artery taken just above the thumb and 4 cm. below the styloid process of the radius may be regarded as normal, although the radial artery examined 8 cm. above the styloid process shows distinct, but not excessive overgrowth of the intima, this being considerably less than that seen in the ulnar artery.

Right brachial plexus.—The brachial artery and the nerve fibres ap-

pear to be normal by Weigert's hæmatoxylin stain and the ammonia carmine, and the degeneration is unimportant by the Marchi method.

A vein taken from the back of the right hand shows some proliferation of the intima.

Left upper limb.—A small portion of the radial nerve and a portion of the brachial plexus were the only tissues of this extremity placed at my disposal for study.

The left radial nerve at a point unknown to me shows as distinct a diminution of nerve fibres and proliferation of the endoneurium as does the right radial 6 cm. above the styloid process.

The intima of the left brachial artery is slightly thickened. The nerve bundles of the plexus by the ammonia carmine stain are normal and yet the Marchi method shows a very intense degeneration of the myeline, which must have been recent—a greater degeneration indeed than is seen in any other nerve except perhaps the right radial. In the left brachial plexus in certain nerve bundles are a few areas in which no nerve fibres exist and only loose fibrous connective tissue is found. These areas are sharply defined from the surrounding nerve fibres of the bundle. In one bundle two such areas are seen. It is impossible to say whether these are imperfections in the original development of the tissue or the result of degeneration of nerve fibres.

Muscle.—The first interosseous muscle of the right hand is not much altered. The muscular fibres are nearly normal in size. The larger intramuscular nerve bundles are wonderfully well preserved, although the smaller bundles show some proliferation of the endoneurium. The intima and media of some of the small vessels within this muscle are thickened.

The skin from the back of the hand just above the fingers has lost the epidermis and the papillæ of the cutis vera are flattened.

Spinal cord.—Sections were taken for microscopical study from the lower cervical and upper thoracic regions and were found to be normal. Nissl's method could not be employed as the spinal cord had been put in Müller's fluid. Some of the motor cells of the anterior horns are vacuolated, but the cells have normal processes and do not appear to be atrophied. Cells found in the area corresponding to Clarke's columns are not atrophied, and so far as can be determined by the carmine stain they are normal. The nuclei in some are eccentric.

The medulla oblongata is normal. The brain by macroscopical examination is normal.

The lesions in this case in brief are as follows: The central nervous system is normal. The nerves in the distal portion of the right hand near the metacarpo-phalangeal articulations are much altered, especially the radial; the ulnar less distinctly so. The right median nerve is slightly altered at about 8 cm. above its terminal portion in the stump of the hand, and the right radial 6 cm. above the styloid process is somewhat degenerated. The nerves could not be studied at higher levels, as the tissue necessary for this was not in my possession. The right brachial plexus is normal. The ulnar nerve is normal except in its most distal portion where the alteration is slight.

The arteries of the right upper limb are diseased in some parts. The ulnar artery near the wrist shows the greatest amount of thickening of the intima and at one portion an organized thrombus is found. The right radial artery shows some proliferation of the intima.

The brachial plexus of the left side gives evidence by Marchi's method of intense recent degeneration, but this is not seen by the ammonia carmine stain. The intima of the left brachial artery is slightly thickened. The left radial nerve is not entirely normal.

It does not seem probable that the vascular changes alone could have produced the peculiar trophic lesions of the right upper limb. Even the organized thrombus of the right ulnar artery had not caused degeneration of the accompanying nerve, and the changes in the right radial artery were not very important. The slight alteration of the ulnar nerve in its most distal portion was probably the result—as was the alteration in the other nerves of the hand—of spontaneous amputation and gangrene, and not of thrombosis of the ulnar artery. The veins had not escaped. The nerves of the right upper limb were seriously diseased only at their terminations near the gangrenous area, except perhaps the radial which was diseased at least 6 cm. above the styloid process of the radius. It is questionable whether so extensive lesions can be explained by so slight degeneration of nerves.

Where peripheral gangrene occurs and the vessels and nerves of the gangrenous limb are found diseased several questions are at once suggested:

1. Was the gangrene caused by the endarteritis obliterans?
2. Was the gangrene caused by the degenerative changes in the nerves?
3. Was the degeneration of nerves and vessels the result of the gangrene?
4. Were the nerve lesions the result of vascular disease?
5. Was the degeneration of the vessels produced by the changes in the nerves?

These questions can be best answered by a study of the cases reported in literature, and they will be considered seriatim.

Raynaud⁵ began one of his papers on symmetrical gangrene with a quotation from a work of Victor François written in 1832: "Everything concerning spontaneous gangrene is in a state of distressing uncertainty." These words are not quite so true as they were at the time they were first written, but much of this "distressing uncertainty" still remains. Cases of gangrene in which the cause cannot be determined are still reported as shown by an interesting paper by McFarland.⁶

1. *Can gangrene be caused by endarteritis obliterans?*

Many cases have been reported in which obliterating endarteritis was believed to be the cause of gangrene. The description of the "gangræna ex endarteriitide hyperplastica" as given by Billroth represents the disease as beginning with prodromal symptoms lasting many years, viz., disturbance of circulation, cyanosis of the limbs, a sensation of cold and weight, paræsthesia, and inability to stand long or walk far. The gangrene is produced by a slight cause and is usually moist.

Von Winiwarter⁷ concluded from his examination of several cases of so-called primary, spontaneous gangrene that the underlying cause is an endarteritis terminating in complete closure of the affected vessels. Zoege von Manteuffel⁸ finds that this form of endarteritis is the result of successive deposition and subsequent organization of layers of thrombi,

⁵ "On Local Asphyxia and Symmetrical Gangrene of the Extremities," by Maurice Raynaud. Translated by Thomas Barlow. London, 1888. The New Sydenham Society, vol. cxxi.

⁶ *Transactions of the College of Physicians of Philadelphia*, 1898, 3. s., xx, p. 160.

⁷ *Arch. f. klin. Chirurg.*, 1878, xxiii.

⁸ *Deutsche Zeitschr. f. Chir.*, 1898, xlvii, p. 461.

so that finally the lumen of the vessel becomes filled with vascularized connective tissue, and Hoegerstedt and Nemser⁹ believe that in general thrombosis participates in a similar way in the production of obliterative endarteritis. Haga¹⁰ in his interesting paper on spontaneous gangrene describes and pictures obliterative endarteritis, which he believes to be of syphilitic origin, as a cause of this disease. The association of gangrene with the group of symptoms called "intermittent claudication," studied by Charcot, Goldflam, Erb, and others, and shown to be dependent on arterial disease, is well recognized. It is not to be doubted, therefore, that gangrene may be caused by endarteritis obliterans, nor is it difficult to understand why this should be so.

Arterial thrombosis, with or without pre-existing arterial disease, is a demonstrated cause of senile gangrene, and may be the cause of the gangrene occurring occasionally as a complication or sequel of infectious diseases, particularly in influenza, typhoid and typhus fevers.¹¹

2. *Can gangrene be caused by degenerative changes in nerves when the blood vessels are healthy?*

The idea that gangrene may be due to diseases of the nervous system alone without any vascular disease is not new. Raynaud¹² refers to the thesis by Zambaco¹³ in which this view was expressed. The paper of Pitres and Vaillard¹⁴ is often quoted in support of the possibility of gangrene resulting from degeneration of nerves. These writers reported two cases in which symmetrical gangrene of the feet occurred, and the arteries and veins were normal. The nerves of the lower limbs were much diseased below the knees, but not above. Those of the upper limbs were normal. Dehio¹⁵ in criticising this paper states that the writers have not proven that gangrene may result from neuritis without vascular disease. Dehio, I think, is quite right in this criticism. The case does seem to show that endarteritis may be absent in gangrene, but the finding of nerve lesions and gangrene in the same limb does not prove that the latter is the result of the former. Both conditions may

⁹ *Ztschr. f. klin. Med.*, 1896, xxxi, p. 130.

¹⁰ Virchow's *Archiv*, 1898, clii, p. 26. Since the completion of this article C. Sternberg's paper (*Virchow's Archiv*, 1900, clxi, p. 199) has appeared with a full consideration of the relation of obliterating endarteritis to spontaneous gangrene.

¹¹ See Welch, Article "Thrombosis and Embolism" in Allbutt's *System of Medicine*, vol. vi, p. 178. London and New York, 1899.

¹² Loc. cit.

¹³ Paris, 1857.

¹⁴ *Arch. de phys. norm. et path.*, 1885, 3. s., v, p. 106.

¹⁵ *Deutsche Zeitschr. f. Nervenheilk.*, 1893-4, iv, p. 1.

result from a common cause, or the gangrene may produce the degeneration of the nerves, as will be mentioned presently. In the report of a necropsy in a case of symmetrical gangrene Raynaud¹⁶ says that the results obtained by himself were absolutely nil in so far as the circulatory system was concerned, so that without Pitres and Vaillard's case we have known for many years that gangrene may occur when the vessels are normal.

Dejerine and Leloir¹⁷ in reporting two observations of gangrenous eschars of the skin, in which they found the nerves diseased, have collected most of the evidence existing at the date of their publication in favor of the occurrence of gangrene as the result of disease of the nervous system. The possibility that neuritis may produce gangrene must, I think, be admitted, although there is not agreement of opinion among authorities as to the interpretation of the experimental and clinical data adduced in support of this view.

3. *Does gangrene cause alterations of vessels and nerves?*

Lapinsky¹⁸ has recently discussed this question quite fully and I cannot do better than to refer to his papers. The investigations of Hodson, Friedländer, Cornil and Ranvier, Ivanowski, Ziegler and others have shown that chronic inflammation has an injurious effect upon the vessels in the neighborhood and causes peri- and end-arteritis. Lapinsky noticed these changes of the vessels in some cases of his own in and near the gangrenous areas.

In reference to the nerves he says that the importance of local gangrene and suppuration in the production of changes in the nerves of the diseased limb has often been discussed without great weight being attributed to those conditions as etiological factors. He quotes a number of cases in which gangrene was found and was not believed to have caused degeneration of nerves, and he attributes no importance to the local gangrene in the production of the degeneration of the nerve stems in cases of his own. It is well known that ascending neuritis from a suppurating wound is of very rare occurrence.

Pitres and Vaillard allude to the fact that nerves passing to a gangrenous area do not necessarily show degeneration, and they say that this was recognized by Vulpian in 1866 and later by Dejerine and Leloir. However, it is probable that in some cases gangrene does cause alteration of nerves, possibly through alteration of the blood-vessels.

¹⁶ Loc. cit.

¹⁷ *Arch. de phys. norm. et path.*, 1881, 2. s., viii, pp. 989 and 391.

¹⁸ *Deutsche Zeitschr. f. Nervenheilk.*, xv, p. 364.

4. *Does degeneration of blood-vessels produce changes in the nerves of the same territory?*

In studying nerves for degenerative changes it is not sufficient to examine sections taken at a distance from the peripheral ends of the nerves. The increase in the degree of degeneration of nerve fibres towards the periphery was observed by Mannkopf in 1878 in a case of embolism of the popliteal and crural arteries.¹⁹ The greater alteration of peripheral ends of nerves has also been seen by Hans Gudden²⁰ and other investigators. The importance of the recognition of this fact is seen, for example, in a case of spontaneous gangrene reported by C. Sternberg.²¹ The sciatic nerve of the diseased limb did not contain degenerated nerve fibres but the vessels were much altered. I am unable to determine from the report of this case whether or not the peripheral ends of the nerves in the amputated limb were studied.

Schlesinger²² says that the primary nature of the vascular disease and the secondary nature of the neuritis are not recognized by all, but he thinks that the vascular alteration occurs first. He reports a case in which pain in the feet and livid discoloration of the feet and hands were followed after some months by gangrene of the left foot. The left foot was amputated and the arteries and veins of the nerves within it were much thickened. The nerve fibres were normal in many bundles but in most they were more or less altered and the connective tissue of the nerves was proliferated. He thinks that without doubt the vascular degeneration occurred before the degeneration of the nerve fibres in this case, and he seems to have based this opinion chiefly on the clinical signs, and yet the disease began with pain in the feet as well as with livid discoloration.

When closure of an artery occurs the degeneration of the nerves may be only in the part below the thrombus, as seen in cases studied by Lapinsky. It is not necessary to quote many examples of this. We can accept without dispute the statement that a nerve speedily degenerates when its blood supply is abruptly cut off. Lapinsky says that in cases of acute ischæmia he found the changes of the nerve fibres more marked towards the distal ends where the effects of closure of the arteries were most felt.

The nerve changes are not so perceptible in chronic vascular disease,

¹⁹ Cited from Lapinsky.

²⁰ *Arch. f. Psychiatrie*, 1896, xxviii, p. 643.

²¹ *Wiener klin. Wochenschr.*, 1895, viii, pp. 650, 687.

²² *Neurologisches Centralblatt*, 1895, xiv, pp. 578, 634.

and according to Lapinsky they have been seen in only comparatively few cases. In some cases the changes of the nerve fibres were very slight and occurred only in certain areas; in other cases the nerve fibres were well preserved and the connective tissue about them was proliferated; in still other cases the nerves were perfectly normal. Lapinsky has collected the reports of a number of cases from the records bearing on this subject. He observed 8 cases of vascular disease; in 7 of these the arteries of one lower extremity were affected, and in one the arteries of both extremities were diseased, and gangrene developed in the part imperfectly nourished. The connective tissue of the nerves was increased in all the cases and this was especially true of the endoneurium.

In the case reported in the present paper the ulnar nerve showed no degeneration as a result of the thrombosis of the ulnar artery.

Joffroy and Achard²³ seem to have been the first to describe neuritis of vascular origin. In a case of neuritis they found that the most pronounced lesions of the vessels were associated with the most pronounced lesions of the nerves, and from this they concluded that the degeneration of the nerves was due to the thickening and obliteration of their nourishing arteries. Neither this case nor the one published by Dutil and Lamy²⁴ establishes beyond question the vascular origin of neuritis although such an origin seems very probable. Dutil and Lamy say that in their case the parallelism existing between the vascular and nervous lesions justifies attributing the degeneration of the nerves to the thickening and obliteration of their nourishing arteries.

5. *Does degeneration of nerves cause alteration of the vessels in the same territory?*

Bervoets²⁵ claims to have demonstrated that cutting nerves causes thickening of arteries in the same territory, and he believes that he has demonstrated that neuritis is a cause of endarteritis. A. Fraenkel^{25a} obtained similar experimental results, whereas C. Sternberg^{25b} had only negative results. Czyhlarz and Helbing^{25c} find an explanation of this discrepancy in their observation that changes in the vessels following experimental lesions of nerves occur only when ulcers result from the operation. Lapinsky²⁶ has collected from the records a large amount

²³ *Arch. de méd. expér.*, 1889, i, p. 229.

²⁴ *Arch. de méd. expér.*, 1893, v, p. 102.

²⁵ Over spontaan gangreen, etc., Nykerk, 1894.

^{25a} *Wiener klin. Woch.*, 1896, ix, pp. 147, 170.

^{25b} *Loc. cit.*

^{25c} *Centralbl. f. allg. Path. u. path. Anat.*, 1897, viii, p. 849.

²⁶ *Zeitschr. f. klin. Med.*, 1899, xxxviii, p. 223.

of evidence in support of the neuritic origin of endarteritis, and he concludes that these vascular disturbances may be of several varieties. In some cases the lumen is enlarged in the vessels of the territory in which the diseased nerves lie, and this part of the body becomes hyperæmic and its temperature is raised. The vessels may become broadened and lengthened and tortuous. In some cases the nutrition of the vascular walls is affected, as shown by local œdema and occasionally small hæmorrhages in the distribution of the diseased nerves. In some cases anatomical changes in the vessels have been found.

Lapinsky refers to a number of clinical cases in which œdema or redness and increase of temperature followed injury or disease of nerves. This causes us to think of erythromelalgia, inasmuch as this redness and increase of temperature were found in later stages of neuritis as well as in the early. Alteration of nerves and vessels was very marked in a case of erythromelalgia reported by Dr. S. Weir Mitchell and myself.²⁷ Lapinsky refers to a number of cases in which changes in the walls of the vessels were believed to result from neuritis. He reports two cases in which disease of the walls of the vessels developed in the distribution of diseased nerves. He believes that the disease of the nerves causes a loss of tonicity and elasticity in the walls of the vessels and a disturbance in the nutrition of the vessels; the enlargement of the lumen, the increased intravascular pressure and the slowing of the blood current lead to further changes.

I have referred elsewhere²⁸ to the views of Thoma. Thoma has shown that when the lumen becomes too great in proportion to the amount of blood flowing through it, as for example after amputation, a compensatory connective tissue thickening of the intima occurs and the proper relations are restored. He has shown that neuritis produces a similar change in the vessels.²⁹ He studied the soft tissues taken from both temples in a case of left supraorbital neuralgia. More or less hyperæmia occurred in the painful area at the time of the attack. He found that the arteriosclerosis in the area of the supraorbital neuralgia was considerably greater than in the corresponding area on the other side. Thoma had acquired so extensive a knowledge of the vascular system that he was able to name most of the large arteries when transverse microscopical sections of them were shown to him. It seems that the vasomotor change caused by the pain in Thoma's case led to this thickening of the intima.

²⁷ *Amer. Jour. Med. Sciences*, Jan., 1899, cxvii, p. 1.

²⁸ *Ibid.*

²⁹ *Deutsches Arch. f. klin. Med.*, 1888, xliii, p. 409.

More recently this subject has been again studied by Lapinsky.³⁰

Although the evidence, especially on the experimental side, is conflicting, there is support for the view that degeneration of nerves may cause degeneration of vessels.

From the preceding statements and review of the evidence relating to the association and correlation of arteritis, neuritis and gangrene it may, I think, be stated:

1. Gangrene may be caused by endarteritis obliterans.
2. Alteration of nerves alone without alteration of the vessels is believed by some to be a cause of gangrene. We need probably more evidence before this conclusion can be definitely accepted.
3. Gangrene may cause degeneration of the vessels, especially of the portions near the gangrenous area.
4. Gangrene is less liable to cause degeneration of nerves except of the portions within or near the gangrenous areas.
5. Sudden closure of blood-vessels causes degeneration of the nerves nourished by these vessels, unless an adequate collateral circulation is promptly established. If the vascular disease is of a chronic type the nerves may escape, at least for a time, but do not always do so, the result doubtless depending upon circulatory conditions which vary in different cases.
6. Degeneration of nerves is a possible, but not thoroughly demonstrated, cause of degeneration of the blood-vessels.

I have noticed in this and other cases of arterio-sclerosis a multiplication of the elastic membrane. This is probably a new formation. It has been regarded by some as merely a separation of the layers of the previously existing elastic membrane, but this explanation is not satisfactory for all cases. In order to furnish so much new elastic tissue the old must have become very much thickened. I have found an elastic membrane in the organized thrombus of the case A. A., and the thrombus was here separated by a clear space from the proliferated intima. It probably represents here newly-formed tissue. Similar views are held by Dmitrijeff³¹ and others.

³⁰ Lapinsky, *Arch. de méd. expér.*, 1899, xi, p. 109.

³¹ Ziegler's *Beiträge*, 1897, xxii, p. 207.

The case of A. A. was not a typical one of Raynaud's disease but it bore certain resemblances to it as Dr. Sinkler pointed out. Local syncope or local asphyxia is not mentioned in the history. The affection was of the distal parts of both upper extremities, in which papulovesicles were important features. Dr. Sinkler describes the sloughing process as first a discoloration of the skin, involving perhaps one-half the surface of the finger; this portion of the finger rapidly became black, then dry, and the slough was thrown off leaving a granulating surface. This account is not unlike that of a case of Raynaud's disease. ³²Monro, in his excellent monograph on Raynaud's disease, says that in less than two per cent of the cases of this disease gangrene alone is mentioned, and a careful perusal of these cases makes it almost certain that there was a stage of asphyxia. In the same proportion of cases, syncope and gangrene alone are mentioned, but in the majority of these asphyxia also was probable. This case A. A. could be considered at most only as an atypical one of Raynaud's disease, but it may have a similar etiology.

There is a class of trophic diseases having certain resemblances to one another but still with distinctive features. Erb³³ compares Raynaud's disease with intermittent claudication. The two disorders resemble one another in the paræsthesia and pain, in the vasomotor disturbances, in the cutaneous gangrene of the fingers and toes, and in the occasional mutilation. In Raynaud's disease neurasthenia, even psychopathies, may occur; the fingers are chiefly implicated; the gangrene is usually limited to the superficial layers of the skin; the symptoms are paroxysmal but not so intermittent as in intermittent claudication and do not depend directly on the use of the limbs. In Raynaud's disease marked changes in the vessels (arteriosclerosis, absence of pulse) and severe gangrene have not been observed. After making these distinctions, Erb refers to Dehio's findings of endarteritis and phlebitis in a case of Raynaud's disease, and says that after all there may be a closer resemblance between Raynaud's disease and intermittent claudication than has been supposed.

³² T. K. Monro, Raynaud's Disease. 1899.

³³ *Deutsche Zeitschr. f. Nervenheilk.*, 1898, xiii, p. 1.

Angiosclerosis, according to Erb, is manifested clinically in a variety of forms. Simple or obliterating arteriosclerosis without nervous symptoms causes senile gangrene or simple spontaneous gangrene; the combination of obliterating arteritis with symptoms of vasomotor irritation and of sensory and motor disturbance causes the intermittent lameness; the combination of symptoms of vasomotor paralysis (possibly of irritation of the vasodilators) with sensory irritation and obliterating arteritis is possibly the cause of erythromelalgia; the combination of obliterating arteritis with vasomotor and trophic and nervous symptoms may be the cause of Raynaud's disease; the combination of arteritis with intense degeneration and inflammation of the nerves causes the angiosclerotic neuritis of Joffroy and Achard, Dutil and Lamy, and Schlesinger; the vasomotor and sensory irritation without the endarteritis causes acroparæsthesia. It is to be noted that in these diseases a functional disturbance in addition to an organic one is common, and in reading the clinical report of the case A. A. as given by Dr. Duhring and Dr. Sinkler I am impressed by the fact that the disturbance was certainly in large part functional.

The interpretation of the lesions found in the case A. A. is difficult. The woman had stigmata of hysteria and probably had Graves' disease. Destruction of nerves near the seat of spontaneous amputation is nothing more than one might expect as a result of the amputation and suppuration. This degeneration decreased upward quite rapidly, and 6 cm. above the styloid process of the radius was slight in the radial nerve except by the Marchi method and was not very evident in the ulnar or median nerve at parts only a short distance from the seat of spontaneous amputation. The vascular disease seems hardly sufficient to explain the loss of the fingers. The ulnar artery from the amputated limb was nearly closed at one part by an organized thrombus, but this had not caused degeneration of the accompanying ulnar nerve, and it is hardly reasonable to suppose that it had caused such serious changes as the loss of the fingers. The alteration of the radial artery was certainly insufficient to explain the symptoms. The right brachial plexus was normal and there was no evidence here of ascending neu-

ritis. The alteration of the left brachial plexus as seen by the Marchi method is difficult to explain. The appearances were those of recent degeneration. Shall we believe that trophic lesions would have reappeared in the left upper limb if the patient had lived longer, or shall we believe that the findings were artefacts? The left radial nerve was as much altered as the right radial 6 cm. above the styloid process of the radius. This alteration may have been due to the serious lesions that had formerly existed in the left upper limb. Dr. Sinkler regarded the case as a trophonurosis dependent upon an hysterical diathesis. There is much to be said in favor of this opinion, but there was nevertheless neuritis of high degree in the periphery of the right upper limb, moderate degeneration of nerves several centimetres above the wrist of the same limb, and some vascular change in the amputated part; and the radial of the left upper limb was diseased. The question as to whether these lesions were primary or secondary I do not think can be positively determined. I have attempted to show that they might have been either. At all events the condition in A. A. was not the result of any distinct lesion in the central nervous system. Functional disturbances in the circulation of the peripheral ends of the limbs, in connection with the organic changes, probably increased the peculiar lesions in the case A. A. Her relapses seemed to depend to some extent on functional disorder.

Dehio³⁴ examined fingers that had been amputated on account of Raynaud's disease and found in these endarteritis, endophlebitis and degenerated nerves, but he only had about 1 cm. of normal tissue above the gangrenous area for his investigation. He, too, was uncertain whether the vascular sclerosis preceded the gangrene or vice versa.

The microscopical examination in the case I report is valuable on account of the extreme rarity of a case of this character with necropsy. It would be most unscientific, however, to be too positive in the interpretation of the lesions, although these were of a definite character, as I have already shown that there is at present considerable difference of opinion concerning the explanation of similar or identical lesions in cases of gangrene.

³⁴ *Deutsche Zeitschr. f. Nervenheilk.*, 1893-4, iv, p. 1.

Dr. Duhring in his two papers in which he reports clinically the case A. A. refers to several similar cases in the literature.³⁵ A brief review of some of these may be of interest and of assistance in judging of the etiological value of the findings in the case A. A.

Doutrelepont's³⁵ case was one of multiple gangrene of the skin over a large portion of the body, associated with vesicles and following the penetration of a needle beneath the left thumb in a hysterical woman. The spinal cord and nerves examined in this case were found to be normal, and the results of the necropsy did not in any way explain the disease.

The case reported by Kopp³⁶ resembles that reported by Doutrelepont. In Kopp's case the lesions were observed on the left breast, left forearm and left thigh. The neurotic nature of the affection Kopp believed was shown by the unilaterality, the acute development of the lesions in groups, the typical course, and the accompanying neuralgia. An ulcerating keloid on the left hand which developed in the scar of a burn, he thought might have caused ascending neuritis and implication of the spinal cord. Kopp describes the case as one of multiple neurotic cutaneous gangrene.

Galton's³⁷ patient was a girl of seventeen who had suffered from fits of an epileptic nature, brought on by a fright at school. Patches of redness followed by blebs appeared on the left wrist, hand and arm shortly after she had chopped off the distal phalanges of the index and ring fingers and cut through the middle phalanx of the middle finger. The eruption was peculiar from the rapid way in which it spread. Sometimes within a quarter of an hour the whole hand and arm would be covered with large blebs which would burst and discharge. The circulation seemed very feeble. At one time a crop of vesicles appeared on the left leg. Galton attributed the lesions to a reflex irritation.

Kaposi's³⁸ patient was a girl of twenty-two years, who had injured her right middle finger by a nail. The part was bound in iodoform. A few days later vesicles appeared on the dorsal surface of this finger and extended and affected the back of the hand and forearm. These vesicles were accompanied by a sensation of burning in the part. Other cutaneous surfaces of the body were attacked. Kaposi did not believe a

³⁵ *Vierteljahresschrift f. Dermat. u. Syphilis*, 1886, xiii, p. 179, and *Arch. f. Dermat. u. Syphilis*, 1890, xxii, p. 385.

³⁶ *Munch. med. Wochenschr.*, 1886, p. 665.

³⁷ *British Med. Journal*, 1891, i, p. 1282.

³⁸ *Wiener klin. Wochenschr.*, 1890, p. 425.

neuritis existed but thought that an ascending irritation was present and caused redness, exudation and vesicles, and that this irritation extended to the spinal cord. He thinks his case resembled Doutrelepont's, and that it was an expression of hysterical irritability of the vasomotor system, analogous to cases of herpes zoster gangrenosus and of zoster gangrenosus hystericus. No gangrene was observed in Kaposi's case. He calls his case "pemphigus neuroticus hystericus."

Bayet³⁹ describes a condition known as disseminated cutaneous gangrene. He speaks of it as a very rare affection. Some of the cases have been described, he says, as gangrenous zona, others as pemphigus neuroticus, and others as gangrenous urticaria. The causes of this confusion are the rarity of the affection and the predominance of certain symptoms in different cases; but common to all are the dependence of the lesions on disturbances of innervation and the local evidences of hysteria. Bayet's case is as follows:

A hysterical male, nineteen years of age, burned himself superficially on the anterior surface of the left forearm a little above the wrist. The wound healed at the end of twelve days. Two days after the accident plaques covered with a dry crust appeared on the external surface of the thumb. This crust did not last very long and left a superficial ulcer requiring two months to cicatrize. Within a short time twenty-one ulcers of different sizes, some as large as a franc, appeared on the left forearm. All these ulcers were found on portions of the skin which had not been in contact with the sulphuric acid. These lesions were found later on the hand. Deeply pigmented areas represented the former site of ulcers, and in some of these areas bullæ, containing a sero-sanguineous fluid, appeared. The skin between these lesions seemed to be normal. The case was believed by Bayet to be a multiple gangrene of the skin dependent upon hysteria. He was able to produce a characteristic lesion by suggestion.

Janovsky and Mourek⁴⁰ in a study of multiple cutaneous gangrene report two cases in which vesicles were observed but no necropsy was obtained. They give references to several cases of cutaneous gangrene. Whether these cases of cutaneous gangrene should be classed with such a case as that of A. A. in whom the lesions were chiefly of the character of vesicles is questionable. The etiology in all is obscure.

³⁹ *Annales de dermat. et de syphiligraphie*, 1894, v, p. 501.

⁴⁰ *Arch. f. Dermat u. Syphilis*, 1896, xxxv, p. 559.

DESCRIPTION OF PLATES IV AND V.

PLATE IV.

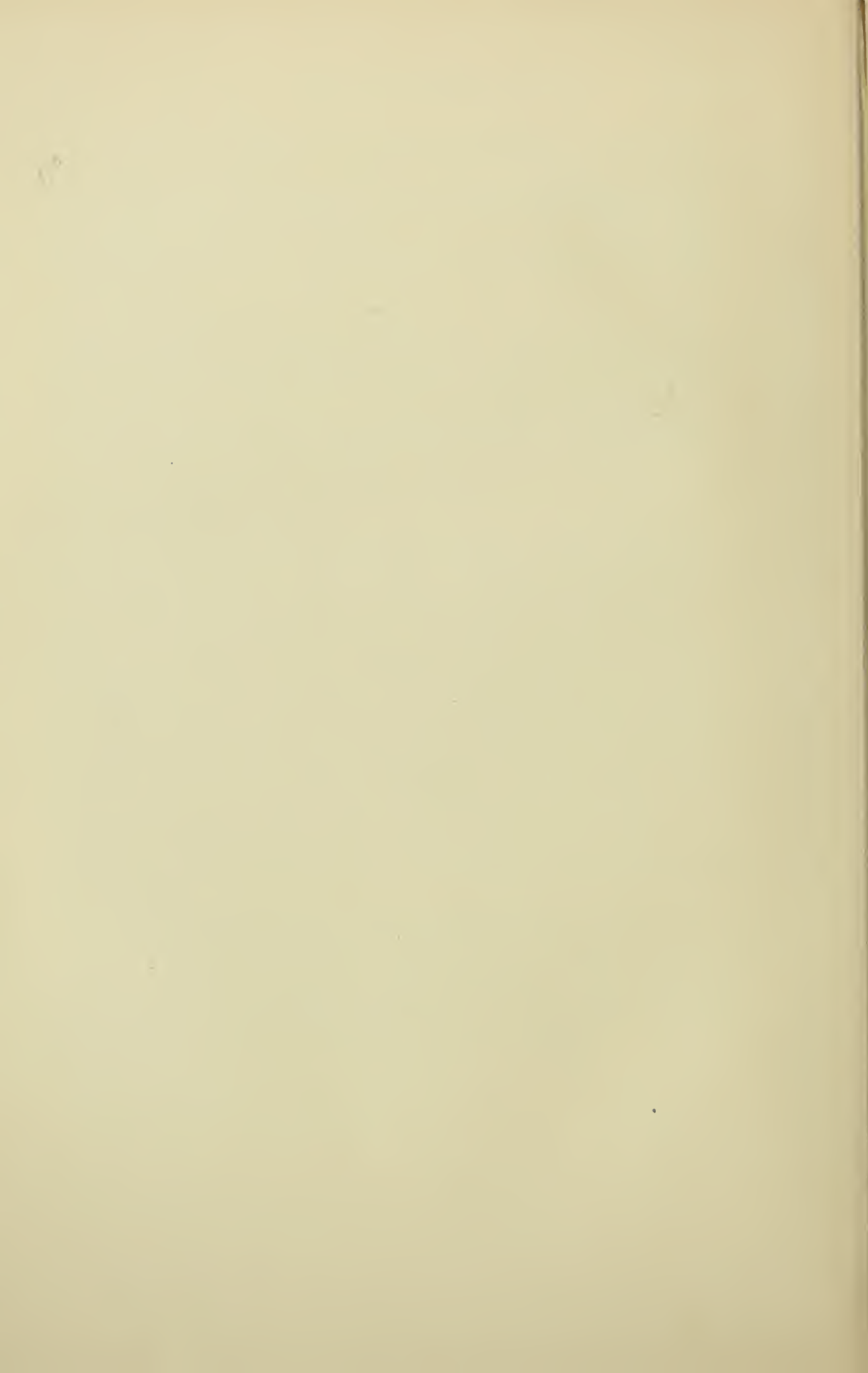
Fig. 1.—Photograph of the amputated part of the right forearm and hand, palmar aspect.

Fig. 2.—An organized thrombus in the right ulnar artery from the amputated part of the limb.

PLATE V.

Fig. 3.—Section of the radial nerve from the right forearm taken 6 ctm. above the styloid process of the radius. The nerve fibres are diminished in number and the endoneurium is proliferated. The degeneration is much less than in the more peripheral portion of the nerve (see Fig. 4).

Fig. 4.—The radial nerve from the right upper limb in its terminal portion. The degeneration of nerve fibres and the overgrowth of connective tissue are extreme.



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FIG. 1.

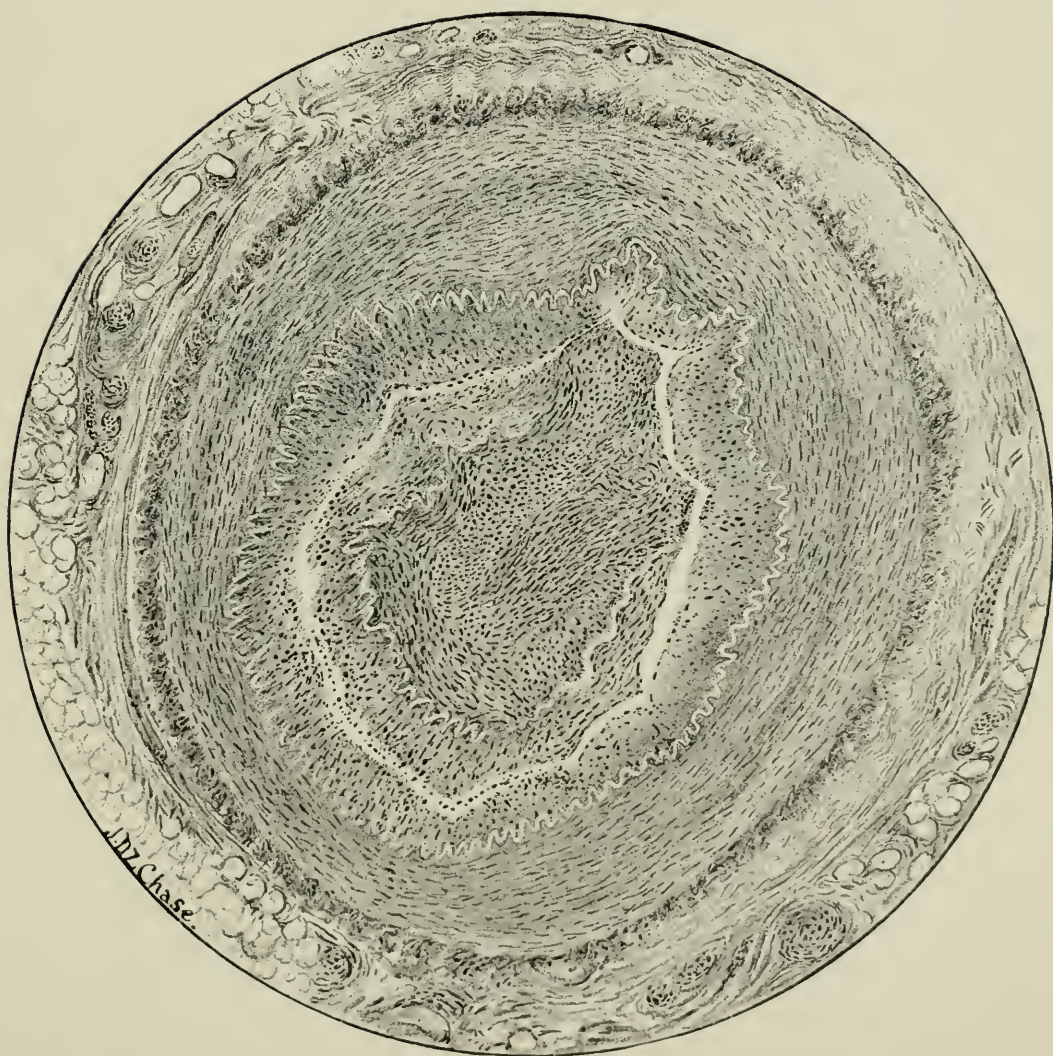


FIG. 2.



FIG. 3.

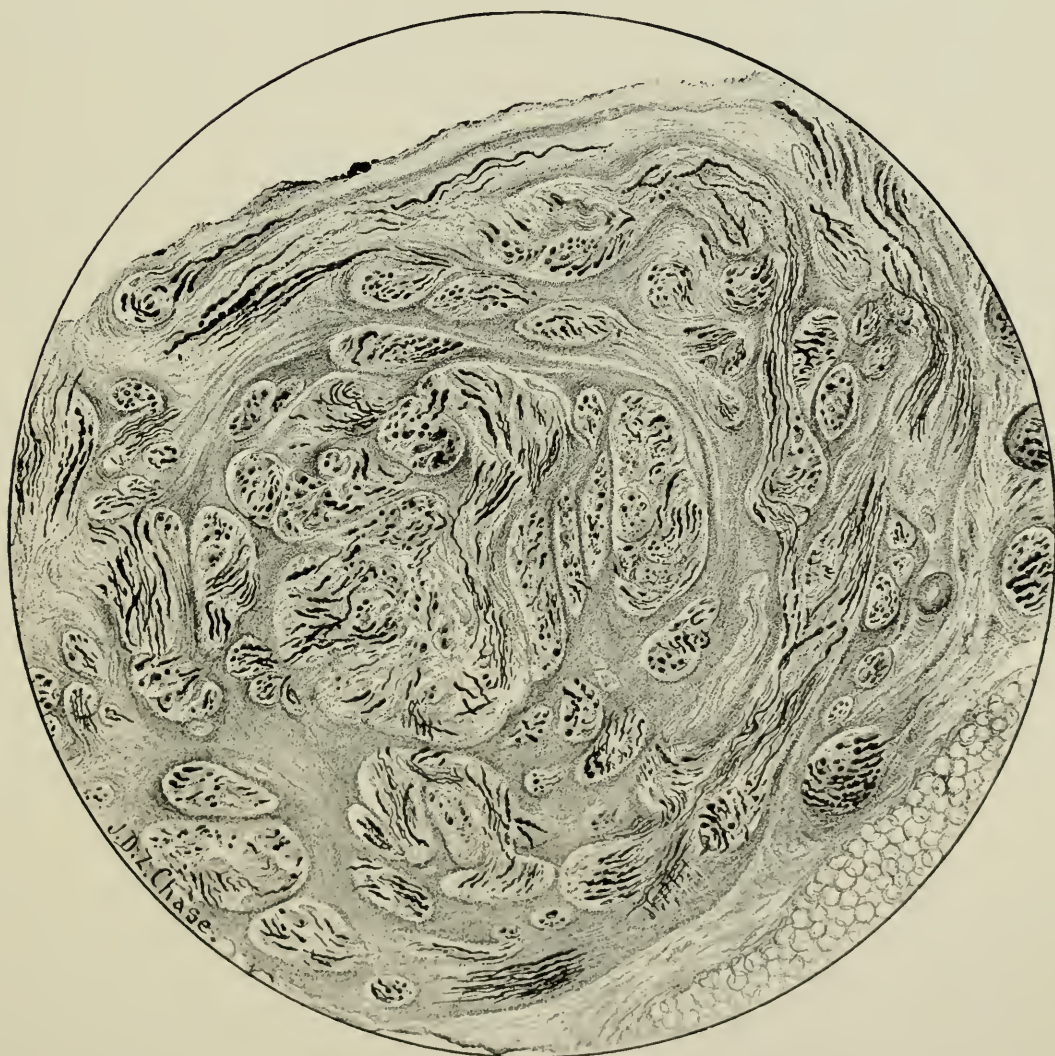
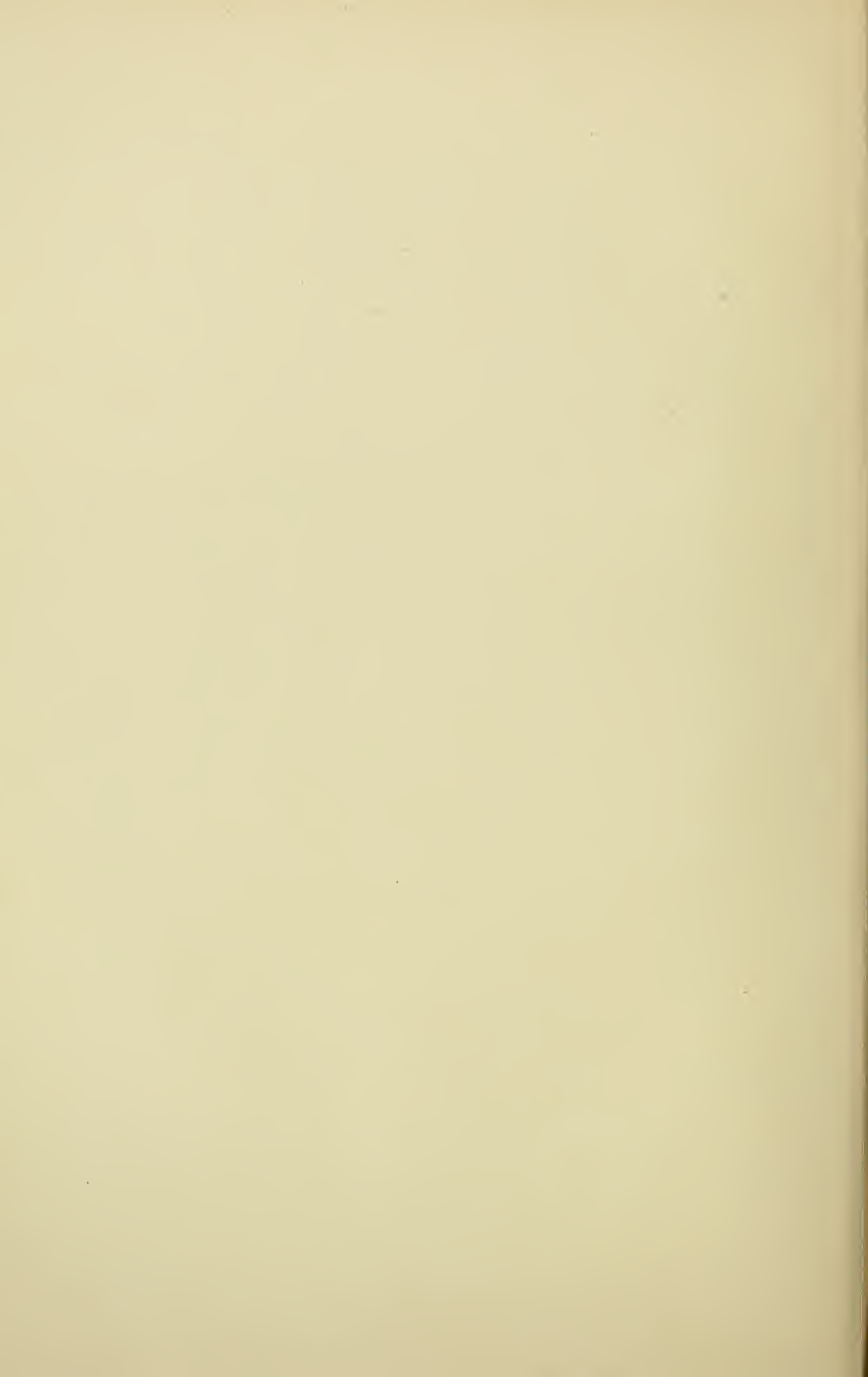


FIG. 4.



AMYLOID SUBSTANCE AND AMYLACEOUS BODIES IN
MULTIPLE SYPHILITIC TUMORS OF THE BONES,
WITH REMARKS ON THE RELATION OF AMYLACE-
OUS BODIES TO AMYLOID SUBSTANCE.

BY W. OPHÜLS, M. D.

[From the Pathological Laboratory of Cooper Medical College, San Francisco, Cal.]

PLATES VI AND VII.

For the pathological material utilized in the following report I am indebted to the courtesy of Drs. L. Newmark and P. K. Brown of San Francisco. The clinical history of the case, which presents some interesting features from a neurological point of view, will be published *in extenso* by Dr. Newmark. In so far as regards our present report it will suffice to say that we learn from it, that 25 years before he came under the charge of Dr. Newmark, the patient suffered from an ulceration of the penis, as evidence of which there remained a large scar. Whether any typical secondary symptoms ever manifested themselves could not be established with absolute certainty. An energetic antisyphilitic treatment, which was given at once, proved to be without any immediate beneficial result.

The findings at autopsy were the following:

Autopsy about 24 hours after death. Strongly built, somewhat emaciated man of middle age. Marked œdema of lower extremities and lower part of trunk; slight œdema of scrotum and penis. Intense cyanosis of face and neck.

On right side of thorax in the axillary line about 15 cm. below axilla is an oblique incision, 4 cm. in length. The bottom of the wound is covered with soft red tissue resembling healthy granulations. In the skin in the neighborhood of the wound, as well as in a number of places on the back, dark purple, almost black nodules of the size of millet-seeds or a little larger are observed, which on incision present the appearances of cutaneous hemorrhages, but are rather distinctly outlined. There

is slight round kyphosis of the upper dorsal and compensatory lordosis of the cervical spine. The processus spinosi of the 2nd and 3rd dorsal vertebrae are infiltrated with reddish-brown, soft masses of tumor. There is a considerable quantity of similar material between the bone and dura mater at this point. The dura mater itself is normal. The infiltration extends far into the bodies of the 2nd and 3rd dorsal vertebrae. The spinal cord is markedly compressed and softened. Ascites, hydrothorax, and hydropericardium are present.

Heart.—Twice the normal size. All cavities equally dilated. Walls of both ventricles thicker than normal (wall of right ventricle averages 5 mm., that of left ventricle about 12 mm.). Valves intact. A few yellow slightly raised spots in the intima of aorta and coronary arteries. The heart muscle is dark brown, very translucent, of waxy appearance. It is extremely firm, so much so that after the opening of the cavities and removal of their contents the heart does not collapse, the walls retaining exactly their former position and shape.

Lungs.—Both apices adherent. From the points of adhesion pigmented scars extend for some distance into the pulmonary tissue. On both sides the visceral and to a greater extent the parietal pleurae are transformed into thick layers of dense white scar-tissue with smooth glistening inner surfaces. Both lower lobes are entirely collapsed; the middle lobe and both upper lobes partly so.

A few lymph glands in the lower anterior mediastinum are enlarged, some of them to the size of small beans. They are very hard to the touch. Cut surface smooth and of a greyish-brown color. Tissue slightly translucent. All of them are surrounded by dense scar-tissue.

Several *ribs* on both sides of the body show large fusiform swellings, some of them as large as goose-eggs. The enlargement is noticeable on all sides but is more marked on the pleural surface, where some of them project from 2-3 cm. above the normal level of the inner surface of the chest wall. On incision they prove to be due to the presence of a reddish-brown, almost pulpy material inside the bone, which has more or less completely replaced the normal structures. The centre of these tumors is darker, the periphery lighter and more translucent, having a waxy appearance. On the outside they, for the most part, disappear gradually into the adjoining healthy tissue, but in some places a somewhat sharper outline is formed by a row of thin spicula of bone. The tumors themselves do not contain any bone. On the inside all of them are covered by the thickened pleura.

Spleen.—One and a half times the normal size; very hard. Cut sur-

face dark purple, smooth. Malpighian bodies visible as greyish-white, translucent dots of the size of a large pin's head. Trabeculæ normal.

Both *adrenals* contain little fat; otherwise they present nothing abnormal.

Both *kidneys* are of normal size; capsule slightly adherent; after its removal the surface appears somewhat granular. The tissue is very hard. Cut surface dark purple; cortex a little cloudy. Glomeruli plainly visible as greyish-red dots. Both renal pelves normal.

Liver.—Small, very hard. Cut surface dark purple; lobules small; centre dark purple; retracts a little from cut surface; periphery lighter.

Pancreas.—In the tail a greyish-red softened area of the size of a hazelnut is observed.

Slight enlargement of mesenteric and retroperitoneal lymph-glands; cut surface pale greyish-white.

In the region between the body and manubrium the *sternum* shows an ovoid enlargement which is more marked on the right side. A cut through the centre of the thickening shows it to be caused by an ovoid tumor, measuring 3 x 1.5 cm. in diameter. The centre of the tumor has a dark, reddish-brown color, the periphery is lighter, greyish-brown, waxy. The entire mass consists of soft, pulpy tissue without any bone. On all sides the tumor is surrounded by a thin shell of bone.

No marked changes in any of the other organs.

Microscopic examination gives the following results: The softening of the pancreas is due to post-mortem digestion. The kidneys exhibit signs characteristic of an old interstitial nephritis with more recent inflammatory changes in the vicinity of some of the medium-sized sub-capsular veins. In spots the epithelium of the convoluted tubules is in a condition of fatty degeneration: in others it is necrotic and partly desquamated. The majority of the renal tubules are filled with granular material or hyaline casts. Many of the vascular loops of the glomeruli and all the medium-sized and small arteries contain amyloid substance in their walls. Many of the arteries show an additional irregular thickening of the intima.

The purple spots in the skin prove to be hæmorrhages into the cutis.

The heart muscle contains large quantities of amyloid substance. The larger part of it is found in the walls of blood-vessels. In those with a muscular coat the outer layers of the media are more particularly affected. A considerable amount of amyloid is found also in the endo- and peri-mysium. Many of the muscle cells are surrounded by a more or less circular sheath of amyloid, which in enlarging has caused first

atrophy, then complete destruction of the cells. By this process several small areas have been completely deprived of muscle cells.

Sections of tissue hardened in 10% aqueous solution of formalin, when tested for amyloid substance, give the following reactions: dilute solution of iodine, no reaction (perhaps due to the hardening fluid); iodine sol. and sulphuric or oxalic acid (5%), bluish-green reaction. Methyl-violet stains the material rose pink.

Enlarged lymph glands from different parts of the body show under the microscope the signs of a chronic catarrhal inflammation with beginning proliferation of connective tissue in the lymph sinuses.

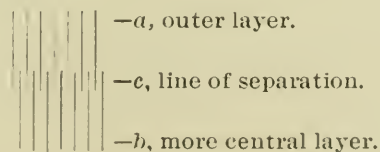
In describing the microscopic appearances of the *sternal tumor* it is best to distinguish 3 layers, a central, a peripheral and an intermediate zone.

1. *Peripheral zone*.—The peripheral layer is rich in cells, with but few connective-tissue fibres between them. The majority of the cells are small and possess round, intensely staining nuclei (Plate VI, Fig. 1). Their close resemblance to lymphocytes and in some instances to plasma cells is striking. In addition to such forms, but fewer in number, we find large cells with vesicular nuclei of oval or more irregular shape. The bodies of the latter are sometimes fusiform (Plate VI, Fig. 1, b), but mostly spherical or polymorphous. The protoplasm composing them is partly granular, but in many instances of a more homogeneous nature (Plate VI, Fig. 1, c; Fig. 2, a). These larger cells have a tendency to coalesce. In a number of such conglomerations the nuclei, and sometimes even the outlines of the cells composing them, are more or less plainly visible (Plate VI, Fig. 1, d; Fig. 2, b). As indicated in the drawings not all the nuclei found in them are vesicular, but some of them are of the lymphocytic type, which shows that the smaller cells also, though to a minor degree, participate in their formation. Eventually the nuclei disappear and there remain irregular, more or less homogeneous masses, some of which are filled with nuclear debris. Around these central masses as a nucleus new material has been deposited in concentric layers and in this way bodies have been formed which in sections, as well as when isolated by teasing, resemble morphologically in every detail amylaceous bodies.

This resemblance is not confined to their external appearance, as is proved by the way in which they react to iodine or methyl-violet. In dilute iodine-solution (e. g. Gram's solution), they stain a dark mahogany brown; the color is generally more intense in the central nucleus than in the peripheral layers. When sulphuric acid is added, there

is, at least in the isolated bodies, no very perceptible change in color, even after 24 hours and more. Methyl-violet stains them a dark blue, which takes a slight purplish tinge within 24 hours. When thin sections of hardened tissue (10% formalin) are first immersed in 5% solution of sulphuric acid and then in Gram's solution the concentric layers in the periphery take a dark brown color, whereas the irregular nucleus shows a bluish-green color. In a similar manner we find that in sections methyl-violet gives the peripheral layers a blue and the central "nucleus" a purple color.

The resemblance to ordinary amylaceous bodies, therefore, seems to be close enough to identify them as such. In very thin sections (4-6 μ) which are stained with methyl-violet and mounted in 20% aqueous solution of acetate of potash, the oil immersion permits the recognition of one more important detail in the structure of the peripheral concentric layers, which is not visible in specimens mounted in Canada balsam. It is then seen, that these layers show most perfect, very closely set, radial markings. Plate VII, Fig. 7, gives only a very inadequate idea of their regularity and delicacy. These markings are the dividing lines between innumerable, very minute, parallel, closely packed needles, of which the concentric layers consist. That they really are needles, and not delicate lamellæ, was shown in one place, where a tangential section had carried off the extreme edge of one of the amylaceous bodies. Here the needles of course appeared, on cross section, as just visible, dark blue dots. In spite of persistent endeavors I have not been able to isolate these needles. They seem to adhere rather firmly to one another and their extreme minuteness in itself would naturally render it difficult to separate them from one another. But even when isolated it would most likely be impossible to make out much about their shape in its finer details. Between the different layers of needles there are darker blue concentric lines, which separate them from one another. These probably represent the tips of the needles from the inner layers reaching in between the bases of those of the peripheral layer somewhat in the way represented in the following diagram:



c naturally appears as a dark line because here the needles are more densely packed together than in *a* and *b*.

The needles of the superficial layers are not all of exactly the same

length. The surfaces of the bodies are, therefore, somewhat uneven, or present a prickly appearance, a fact which may account to some extent for the formation of large giant cells (Plate VII, Fig. 5, a) in their neighborhood. It would seem most probable that these enormous giant cells, which sometimes almost completely envelop the amylaceous bodies, do not furnish any new substance for their enlargement, but on the contrary destroy them. Such an assumption would at least account for the fact that they are frequently situated in excavations in the surface of the amylaceous bodies, so that they would be analogous to the osteoclasts in Howship's lacunae and would furnish another illustration of the destructive influence of these foreign-body giant cells (*Fremdkörperriesenzellen*) upon the material around which they develop.

The "nucleus" of the amylaceous bodies, which stains purple with methyl-violet, is generally more lumpy in character, but in some places even this part of the amylaceous body is found to be composed of densely packed needles, which are arranged in a radial fashion around a central point (Plate VII, Fig. 7, a). In these cases the crystalline needles also stain purple in methyl-violet.

Crystalline formations are not found exclusively in the amylaceous bodies. In a few spots I have encountered isolated large bunches of needles arranged in a radial fashion around a more homogeneous central body; needles and nucleus giving the amyloid reaction with methyl-violet.

Forms represented in Plate VII, Fig. 8, are even more peculiar. In the centre or more towards the periphery of an irregular lump of colloid substance which takes a blue color in methyl-violet, we find a spherical or ovoid cavity containing a rosette of purple needles.

Plate VI, Fig. 4, shows another peculiarity of the amylaceous bodies, which is very marked in our case. They frequently become adherent to one another and in this way large conglomerations consisting of 20 or more of these bodies are formed.

There remains to be discussed briefly one point of some interest and that is: At what stage in the transformation of the conglomerated large cells into the nucleus of amylaceous bodies does the amyloid reaction appear? The specimens show in this regard that the transition of the colloid protoplasm into amyloid material is a slow and gradual one. Even before the nuclei have disappeared, the cell bodies begin to stain a little darker yellow than the surrounding tissue and many of them also show a faint but quite distinct purple color when treated with methyl-violet.

The blood-vessels in the peripheral layers of the tumor are few in number, of small calibre, and their walls are normal.

The peripheral cellular layers, which I have just described and in which the amylaceous bodies are formed, do not form a regular, even zone in the periphery of the tumor, but are present in irregular patches, most of them rather limited in extent, which invariably, however, are found in the periphery.

2. *Intermediate zone.*—The tissue loses to a great extent its cellular character and shows the appearance represented in Plate VII, Fig. 5. It consists of a fine meshwork of connective tissue, the spaces of which are filled with numerous amylaceous bodies isolated or arranged in groups. The larger part of them are surrounded by large giant cells. In some spots in the connective tissue there is an abundant deposit of brown pigment (Fig. 5, b).

In the intermediate zone of the tumor we find, in addition to the amylaceous bodies, thick irregular bands and lumps of ordinary amyloid substance, which appear first in the reticulum of connective tissue. When stained with methyl-violet these bands mostly take a dark purple color throughout, but in all sections are some in which the purple centre is surrounded by a dark blue colloid material.

3. The *centre* of the tumor is composed of fragments of amylaceous bodies and of amyloid bands. The spaces between them are filled with red blood corpuscles. In some places, among the masses of red blood corpuscles, it is possible to follow out for some distance collapsed tubes, with their endothelial walls, which apparently are partly destroyed capillaries that have discharged their contents into the surrounding parts.

All the colloid material in the central parts of the tumor stains with dilute iodine solution brown, which turns to a bluish-green when sulphuric acid is added. With methyl-violet it takes a purple color.

The formation of amyloid substance is not confined to the tumor itself, but is also noticed, although to a minor degree, in the neighboring tissues. Plate VII, Fig. 6, for example, shows a portion of the periosteum under the higher power. The amyloid material is seen to be deposited in and between the bundles of fibres of connective tissue and also in the walls of capillaries. The specimen from which the drawing was taken was stained by Van Gieson's method,¹ which, as Schmidt² has pointed out, brings out clearly the relation of the connective-tissue fibres

¹ In the drawing the bright purple connective-tissue fibres are shown as black lines.

² "Ueber die localen Amyloidtumoren der Zunge," Virchow's *Archiv*, 1896, cxliii, p. 369.

to the amyloid material. A careful study of this specimen and of many others of the same kind shows that nowhere is there the slightest indication of a transformation of connective-tissue fibres into amyloid substance. The latter always appears first in the spaces between the fibres, it thrusts them apart and destroys them, at least to a certain extent, as may be concluded from the presence of short pieces of apparently broken fibres in some of the amyloid bands. There is, however, no evidence of a direct transformation of one into the other.

Not only amyloid substance proper but also a few amylaceous bodies may be found in the tissues, sometimes in the neighborhood of the tumor, but at other times quite remote from it.

Specimens of the tumor of the spinal column which were examined with the microscope had precisely the same appearance as the intermediate zone of the sternal tumor.

The costal tumors also differ very little from the sternal one. The number of amylaceous bodies in them is smaller, but there is comparatively more ordinary amyloid substance and also more fibrous but very little cellular tissue.

Unfortunately it was impossible to make the customary tests for amyloid at the time of the autopsy, which was performed at an undertaker's establishment under unfavorable conditions. By mistake material for microscopic examination was not obtained from the spleen or liver. Nevertheless, the macroscopic appearance of the spleen indicated the presence of amyloid in this organ. In regard to the liver, which showed evidence of a certain degree of cyanotic atrophy, I am doubtful about this point.

The findings, therefore, may be summarized as follows:

Multiple tumors of bones (sternum, ribs, spinal column) with local formation of amyloid substance and amylaceous bodies in them and in the surrounding tissues; amyloid degeneration of kidneys, heart and most likely also of spleen; interstitial and parenchymatous nephritis; hydrothorax, ascites, hydropericardium, anasarca, chronic passive congestion of the abdominal viscera.

In considering the post-mortem findings, first of all the question arises: What is the nature of these tumors?

In looking over the literature at my disposal I find that Hildebrand³ has described a tumor, which, at least in some respects, re-

³ Virchow's *Archiv*, 1895, cxl, p. 249.

sembles very closely those found in our case. The tumor, which was examined by him, was situated in the sternum. It had been removed by König with good results, although at the operation two serous cavities—one pleura and the pericardium certainly, and very likely also the other pleura—were opened. The patient died later at home of a disease apparently not connected with the former presence of the tumor. No autopsy could be obtained. The tumor was of the size of a prune. It did not have any bony capsule, nor was there any bone in the tumor itself. The histological examination revealed the presence of a richly cellular tissue composed mainly of two forms of cells—smaller ones with intensely staining nuclei, and larger ones which took the nuclear stains more faintly. The protoplasm of the latter was granular and stained well with eosin. There were also some giant cells. Some connective-tissue fibres could be made out between the cells. The capillaries were large and numerous. A large number of amylaceous bodies and a good deal of amyloid material in bands and lumps were present in the cellular tissue. The very intimate relation between amylaceous bodies and adjoining cells, which was observed in some spots, led Hildebrand to infer that the amylaceous bodies originated, partly at least, from degenerating cells of the tumor. There is not even an allusion to any syphilitic history either in König's or in Hildebrand's report; presumably therefore there was nothing that pointed to the existence of such an infection. On the basis of his microscopic findings Hildebrand regards his tumor as a myelogenic giant-celled sarcoma (endostales Riesenzellensarcom). But in spite of the great general similarity which exists even in the microscopic findings, in both cases, I am doubtful whether to accept Hildebrand's diagnosis, at least in our case. When we compare the microscopic findings one important difference at once becomes apparent, and that is the presence of a considerable quantity of ordinary connective tissue at least in the inner, and therefore most likely older, layers of our tumors. This characterizes, to my mind conclusively, the tissue composing them as granulation tissue and excludes the possibility of their being sarcomatous. It is possible that Hildebrand was misled in his diagnosis by the presence of only the earliest stages

of granulation tissue in his sections. But this suspicion could not of course be verified without a renewed study of sections from his tumor.

An interesting feature of our case, undoubtedly, is the possibility offered of tracing with comparative ease the mode of formation of the amylaceous bodies present. When we study the literature in regard to their formation in other localities of the body, we find very marked discrepancies between the statements of different writers. In fact the divergences are so striking that one might be inclined to believe that the *modus formandi* is not the same in all localities and may differ even in the same organ according to circumstances.

The material composing these bodies might be furnished in any of three ways: (1) By degeneration of cells or formed intercellular substance; (2) by precipitation from the tissue juices, or (3) by a combination of (1) and (2), part of the material being furnished by degeneration, and part of it by precipitation from fluids.

The second view, viz., that the bodies are merely the result of precipitation from the surrounding fluids, has been frequently connected with the names of Virchow and of Rindfleisch, although in their writings, so far as they have been at my disposal, I have been unable to find any direct statement to this effect. Friedreich⁴ on the other hand undoubtedly regarded the amylaceous bodies, which he discovered in the lungs, as such precipitations. He believed that they were formed from extravasated blood by a peculiar form of coagulation.

More or less in favor of a cellular origin of amylaceous bodies are Ziegler,⁵ Stilling⁶ (these two at least so far as prostatic concretions are concerned), Zahn,⁷ Favre,⁸ and Wichmann⁹ (the last three in a more general way). Ziegler and Wichmann are of the opinion that in the prostate the epithelial cells of the glands are transformed into homogeneous masses by a degenerative process and these, or frag-

⁴ Virchow's *Archiv*, 1856, x, pp. 201, 507.

⁵ *Lehrb. der allgem. Path. und path. Anat.*, 9te Aufl., i, p. 226, Jena, 1898.

⁶ Virchow's *Archiv*, 1884, xcviii, p. 1.

⁷ Virchow's *Archiv*, 1878, lxxii, p. 119.

⁸ Favre, Thèse de Genève, 1879, cited from Siegert, Virchow's *Archiv*, 1892, cxxix, p. 517.

⁹ Ziegler's *Beiträge*, 1893, xiii, p. 487.

ments of such, coalesce to form the amylaceous bodies. Among the authors that have described amylaceous bodies in tumors,¹⁰ there are also two who believe that in their cases these bodies had originated from degenerating cells or that degeneration of cells played at least an important rôle in their formation. One of these, Hildebrand, who, as I said above, was probably led to this view by the close connection sometimes found between degenerating cells and amylaceous bodies, nevertheless could not arrive at a definite opinion as to whether the material composing them was excreted by the degenerating cells or the cells themselves were directly transformed into amylaceous bodies. Langhans,¹¹ who describes a carcinoma of the lung containing amylaceous bodies, believes he has seen in his specimens transitional stages between degenerating tumor cells and such bodies.¹²

Posner¹³ altogether rejects an exclusive view on the subject and states as his belief that amylaceous bodies may be formed not less by degenerative processes from cells than by precipitation from fluids; while Paulizky¹⁴ arrives at the conclusion that in the prostatic concretions the centre, the so-called "nucleus" (Friedreich¹⁵), is a product of degenerating cells, whereas the peripheral concentric layers are precipitations from the surrounding fluids.

The third view, that products furnished by degenerating cells and the tissue juices combine in the formation of amylaceous bodies, is held by Siegert,¹⁶ at least for part of the concretions (*corpora versicolorata*). Ziegler also seems to favor it so far as the formation of amylaceous bodies in the lungs and central nervous system is concerned, while Lubarsch¹⁷ has endorsed it in a more general way for all forms.

¹⁰ For the literature on this subject, see Hildebrand's article, *loc. cit.*

¹¹ Virchow's *Archiv*, 1867, xxxviii, 537.

¹² Langhans mentions in his paper that amylaceous bodies were found only in the peripheral parts of the tumor. This would suggest the possibility that they were formed in the lung before it was invaded by the carcinoma, and that they might have been taken up only later into the growing tumor.

¹³ *Zeitschr. f. klin. Med.*, 1889, xvi, p. 144.

¹⁴ Virchow's *Archiv*, 1859, xvi, 147.

¹⁵ *l. c.*

¹⁶ Virchow's *Archiv*, 1892, cxxix, p. 513.

¹⁷ Lubarsch and Ostertag's *Ergebnisse*, Abth. II: Allg. path. Morph. u. Physiol., p. 200, Wiesbaden, 1895.

Siegert's¹⁸ attempt to solve the difficulties by subdividing the amylaceous bodies into two groups according as they give certain color-reactions (*corpora versicolorata*) or not (*corp. flava*), and postulating a different *modus formandi* for each group, does not appear to be very successful. A subdivision on this basis was first suggested by Virchow long ago, but Lubarsch,¹⁹ although accepting it in a general way, has pointed out some of the difficulties which we encounter, when we try to adhere to it strictly. For myself, I must confess that in studying the amylaceous bodies of the prostate it has been absolutely impossible for me to arrive at any satisfactory classification on these lines. There are so many transitional forms between bodies giving no reaction to solutions of iodine and those with typical amyloid reaction, that it has been impossible for me to find any sharp line of division between Siegert's two classes. Furthermore, when we employ diluted sulphuric acid in addition to the iodine solution, the matter becomes even more complicated, because sometimes bodies which will not react with iodine alone, turn green as soon as sulphuric acid is added, and again, part of an amylaceous body, for instance, the periphery, may show no reaction, whereas in the centre it is quite marked. Similar differences in reaction become apparent, when we use solutions of methyl-violet. On account of these difficulties I do not believe that much can be gained by so artificial a subdivision. The reactions, which at first sight seem to be so important, on closer study lose much of their apparent value and on account of the infinite variations encountered it would seem only natural to conclude that they are not essential but more or less accidental. This conviction has been much strengthened by the study of the amylaceous bodies found in the tumor-like growths in our case, because even the crystalline needles, which were present, in some places reacted to methyl-violet and in others not at all.²⁰

¹⁸ loc. cit.

¹⁹ loc. cit.

²⁰ I have purposely omitted to mention the different views held concerning the formation of amylaceous bodies in the central nervous system, since they are so contradictory as to make a renewed careful study of this subject necessary before any intelligent use can be made of the data which have accumulated in course of time.

My specimens seem to leave hardly any room for doubt, that in our case the central masses of the amylaceous bodies, in a manner similar to that described by Paulizky²¹ for the prostatic concretions, were largely furnished by degenerating cells, whereas the crystalline needles in the periphery are more likely to be precipitations from the surrounding fluids.

When I say degenerating cells, I do not wish to employ the word in its strictest sense. It would perhaps be better to speak of a transformation (metamorphosis) of the cells, because I believe that the colloid material, which accumulates in their bodies and which corresponds so closely to the amyloid material found in other parts of the tumors outside of cells without any connection with a degenerative process in the latter, is largely taken up into the cells from the outside. Such being the case the process has more of the nature of an infiltration, if in the light of recent knowledge we are at all justified in making such a strict distinction between degeneration and infiltration.

It seems that in our case the more irregular central masses, the "nucleus," furnished a point of crystallization for the needles on the outside, although evidence of crystallization may sometimes also be found in the central masses themselves.

The present is not the first observation of an apparent crystallization of amyloid material. Maximow²² found in the liver of a horse, with amyloid degeneration of the viscera an amyloid substance in a crystalline form. Some of the irregular masses of amyloid material were covered with needles somewhat varying in width, which gave a typical reaction with methyl-violet. Maximow found similar needles in the livers of rabbits with experimental amyloid degeneration, although he could detect them only in specimens which were hardened in alcohol and was unable to see them in the fresh tissues. He also claims to have encountered appearances similar to these, although in a much less marked degree, in human livers with amyloid degeneration. On the strength of my own observations I am satisfied that

²¹ l. c.

²² Virchow's *Archiv*, 1898, cliii, p. 361.

Lubarsch's²³ suggestion that what Maximow observed might have been due to post-mortem changes, is not correct; nor do I think that the case of Lindemann,²⁴ which he cites, can furnish a confirmation of his view. In this case peculiar crystals, which did not react to methyl-violet, were found in a specimen of a human amyloid liver, which had been preserved in alcohol for some time. It is quite probable that the appearance of these crystals was due to post-mortem changes, which had taken place in the hardening fluid, but they are plainly entirely different from those which Maximow describes in his article.

Granting, then, that in our case the amylaceous bodies were formed by a genuine crystallization around a "nucleus," such as a small lump of ordinary amyloid substance, we should naturally ask the question, whether the same or at least a similar mode of formation holds true in a general way for all amylaceous bodies. I have confined my studies in this regard to the amylaceous bodies of the prostate, since they are most readily obtained. In these investigations I encountered an obstacle which is difficult to overcome at present. The peripheral concentric layers of the prostatic concretions are not only very much thinner than those found in the amylaceous bodies of our case, but their structure seems to be so much more delicate that even very good immersion lenses do not allow the recognition of details with sufficient accuracy. While it is true that one can make out a faint, very dense radial striation in them, it is nevertheless impossible to assure oneself positively that this radial striation is the effect of rows of densely packed needles. However, I think that the coarser radial striation, which is comparatively rare in the prostatic concretions, but which has been described more frequently in the amylaceous bodies of the lungs, also indicates a structure similar to that found in the large amylaceous bodies of our tumor. In the immediate vicinity of the nucleus quite large crystalline needles arranged in radial fashion may be seen more frequently. Posner²⁵ already has called attention to this fact. He believes them to be

²³ Lubarsch-Ostertag's *Ergebnisse*, Jahrg., iv, 1897, Allg. Path. u. path. Anat., p. 454. Wiesbaden, 1899.

²⁴ *Centralbl. f. allg. Pathol. u. path. Anat.*, 1897, viii, p. 385.

²⁵ loc. cit.

crystals of cholesterin, which can hardly be the case, since according to the reports of later authors they are not soluble in ether-alcohol. It seems to me more probable that they are of the same kind as those found in the specimens from the tumors.

A short while ago I compared the crystalline structure observed in the amylaceous bodies of our case with similar crystallizations observed by others in the ordinary amyloid substance. To do this we must, of course, accept the proposition that both substances, the one which is found in the amylaceous bodies and that which composes the amyloid material, are identical or at least closely related to one another chemically. In spite of the statements to the contrary by a large number of competent observers (von Recklinghausen, Ziegler, Posner, Wichmann, Schmidt and others) I am very much inclined to believe that such is the case. In discussing this question, we must not forget that the mere fact that the formation of amylaceous bodies in certain localities has to be regarded as a semiphysiological occurrence, whereas amyloid substance seems to be found under certain diseased conditions only²⁶ and in other parts of the body, does not in itself constitute a sufficient reason why the material in both cases should not be identical. At least I do not see why in certain localities the production of such a substance should not go on under physiological or at least almost physiological²⁷ conditions, whereas in others it originates in case of disease only.

Again the differences which they frequently show in their reactions to solutions of iodine, methyl-violet and other chemical reagents (osmic acid, carmine [Posner]) cannot be looked upon as rendering their identity impossible, because these reactions are varying and are not quite constant in either the amyloid substance or the amylaceous bodies. This is a generally recognized fact with the amyla-

²⁶ Krawkow, *Arch. f. exp. Path. u. Pharm.*, 1898, xl, p. 195. Lately Krawkow claims to have found a material that is at least very similar to, if not identical with, the amyloid substance, in the normal aorta of horses, in the ligamentum nuchæ of cattle, in the stroma of the spleen of calves and the mucous membrane of the stomach of pigs.

²⁷ Stilling (Virchow's *Archiv*, 1884, xcviii, p. 1) attributes the formation of amylaceous bodies in the prostate to a stagnation of the secretion due to pathological changes in the organ such as hyaline degeneration of the muscle in the neighborhood of the ducts, the *myxangioiditis hyalinosa* of von Recklinghausen.

aceous bodies. In the case of the amyloid substance Virchow very early pointed out the remarkable differences in reaction observed, when this material is treated with dilute solutions of iodine and sulphuric acid. The recent observations on the experimental production of amyloid degeneration have demonstrated, that certain forms, apparently the earlier stages, will give a typical reaction with methyl-violet, but do not react with solutions of iodine, showing that even the simple iodine reaction is not a constant phenomenon. Going a step further we may even assert that sometimes in the very earliest stages the body which is deposited in the tissues, does not give either of the two reactions, for I believe with Wichmann that the careful researches of a large number of observers—Litten²⁸ among the first—leave no room for doubt that the “hyaline” material which is sometimes found with the amyloid substance, or alone in cases in which we might expect to find amyloid, may become transformed in course of time into true amyloid with typical reactions. This assumption is in accordance with the findings in our case, in which lumps and bands of more or less homogeneous material were present, which gave a partial reaction with the methyl-violet; the central parts (probably the older ones) staining purple, the periphery violet. Some of the recent experimental work also seems to be confirmatory of this view. Lubarsch²⁹ found after seventeen weeks in the spleen of a dog, that had received numerous injections of turpentine, several arteries with typical hyaline degeneration; and four weeks later in another piece of the same spleen hyaline and amyloid arteries were demonstrated. He also found hyaline and amyloid arteries at the same time in the spleen of a rabbit. It must be understood, however, that the “hyaline” substance is not found with the amyloid substance frequently enough to justify the conclusion that this primary formation of “hyaline” is a constant occurrence, but such may be the case and, what seems to deserve some attention, the transformation of one into the other takes place without any perceptible morphological changes in the material. On the other hand we know how comparatively easily

²⁸ *Deutsche med. Wochenschr.*, 1887, xiii, p. 517.

²⁹ Lubarsch-Ostertag's *Ergebnisse*, Jahrg., iv, p. 456.

the amyloid substance loses its characteristic reaction. All hardening fluids impair it and tissues which have been hardened in Müller's fluid for some time, give a very imperfect or no reaction at all. Litten³⁰ showed that amyloid substance when brought into the peritoneal cavity of living animals loses its reaction before being absorbed—a point which has been confirmed by Grigorjeff.³¹ In all these cases the macroscopic and microscopic appearances of the substance are not changed in any way.

In view of these facts, I think that we have a right to be a little doubtful whether the presence or absence of the reactions with iodine and methyl-violet indicates a difference in the chemical nature of the body itself, or whether the reactions are not due rather to the admixture of some foreign substance from the outside—a view that, at least in the case of the amylaceous bodies, has been expressed more or less definitely by more than one writer on the subject. But even if there should be a change in the chemical composition, it cannot be a very fundamental one. In this connection, also, we approach the most interesting and much-discussed question whether the staining reactions so commonly employed in the microscopical technique are really all chemical reactions; or if such is not the case, which of them have to be considered as such; but it would lead us too far away from our present subject to enter into a discussion of this point. Even if the change in reaction should indicate some slight chemical alteration, I think it would be better not to over-emphasize the importance of one of these reactions by calling the substance “amyloid,” although of course I understand the historical and other reasons which might be adduced in favor of the use of this term. If we should go back to Rokitansky's term “lardaceous³² substance,” we should be able to unite what is now called “amyloid” and the primary “hyaline,”

³⁰ loc. cit.

³¹ Ziegler's *Beiträge*, 1895, xviii, p. 37.

³² The comparison to lard is not very elegant or striking. It was used by Rokitansky not exclusively for amyloid conditions, but it has been widely adopted by numerous authorities, especially among English writers; nor is it so apt to cause confusion as a new term undoubtedly would. Colloid might be suggested as an alternative, but to my mind it would be best to restrict this term more and more to what P. Ernst has called epithelial colloid (*Virchow's Archiv*, 1892, cxxx, p. 377).

which is now described as an intermediate product in the formation of amyloid substance proper, under one heading and to speak of lardaceous degeneration with or without amyloid reaction—a form of expression, which to my mind has certain advantages to recommend it. In the first place it does not put any stress on the more or less inconstant reactions, and besides would separate the “hyaline” which sometimes precedes and accompanies the amyloid from the heterogeneous substances now comprised under this name. I believe with other authors that only in this way—by trying to eliminate as many substances as possible from this general group—will it be possible to arrive in course of time at a satisfactory definition of what should be called “hyaline.”

Coming back after this digression to our original theme, I think that the above considerations show that neither the differences in the conditions under which they are formed nor the differences in reaction encountered constitute a sufficient reason for a separation of the “amyloid” material proper from the substance of which amylaceous bodies are composed. In fact the frequent similarity in reaction, if it proves anything at all, makes it rather probable that the two substances, if not identical, are at any rate closely related chemically, since, although they may not exhibit these reactions from the beginning in one form or another, both of them may do so in the later stages of their formation. Another strong reason in favor of this conception may be found in a fact which has been already sufficiently emphasized by Hildebrand. In his case and also in our own, “amyloid” substance as well as amylaceous bodies were formed in the same locality simultaneously in such intimate local relation with one another, and both presenting such varying degrees of staining reactions, as to make it difficult to believe that the one body could be very different from the other.

But in spite of all this there remains one important *morphological* difference. Amyloid substance appears in irregular lumps and bands, whereas the amylaceous bodies have a characteristic form and structure, possessing a central nucleus of amyloid or other material (pigment, remnants of disintegrated cells) surrounded by regular con-

centric layers. This remarkable regularity in structure has been dwelt upon by many authors and has led some, Lubarsch among them, to compare the process to a crystallization. After what I have seen in my case I am convinced that the process not only may be likened to a crystallization but really is one, adding in this way a new example to the few already known of a crystallization of albuminous substances—a theme, which I have treated a little more fully in a paper read before the Microscopical Society of San Francisco on November 1, 1899.

Amylaceous bodies and amyloid substance differ from one another to this extent: in the one the material is present in amorphous, in the other in crystalline form, although, as is shown in Plate VII, Fig. 8, crystallizations may occur also inside amorphous lardaceous material.

In regard to the mode of formation of the lardaceous substance I have to add very little. It would be entirely beyond the scope of this paper to review the history of the genesis of amyloid substance, more especially so, since in Wichmann's³³ excellent article "Die Amyloiderkrankung" we have a remarkably able and complete review of the subject. From the study of the case described here in detail, and from subsequent examination of thin sections from several cases of amyloid degeneration I can only confirm with Lubarsch Wichmann's statement, that in "amyloid degeneration" we find no conclusive evidence that the amyloid substance is furnished by a degeneration of cells or of intercellular substances, such as connective-tissue fibres or basal membranes. The material is found from the beginning in the spaces between the formed elements; and Wichmann's and Ziegler's hypothesis that it is derived from stagnating albumin seems to be quite acceptable. In the case of the amylaceous bodies the evidence for their formation from degenerating cells seems to be very much stronger, but, in spite of the errors involved in conclusions by analogy, I should suggest it to be more probable that even here the material is not a mere product of degeneration but rather of the nature of an infiltration from the outside. Nor would such an explana-

³³ loc. cit.

tion exclude the possibility that the disintegrating cell-bodies might furnish part of the albumin which is used in the formation of the lardaceous substance.

The general conclusions reached may be formulated as follows:

The substance of the amylaceous bodies is identical with or at least similar to amyloid substance. In the amylaceous bodies this material is present in crystalline form, in the amyloid substance in amorphous form.

It would be advisable to use the word "lardaceous" instead of "amyloid" and perhaps to include under this term the peculiar form of "hyaline" which sometimes precedes and accompanies the amyloid substance.

DESCRIPTION OF PLATES VI AND VII.

PLATE VI.

Fig. 1.—About 400 x. Specimen stained with Van Gieson's method; mounted in xylol-balsam.

Fig. 2.—520 x. Specimen stained with Van Gieson's method; mounted in xylol-balsam.

Fig. 3.—110 x. Specimen stained and mounted as in Figs. 1 and 2.

Fig. 4.—110 x. Teased specimen from tissue hardened in 10% solution of formalin.

PLATE VII.

Fig. 5.—110 x. Specimen stained and mounted as in Fig. 1.

Fig. 6.—285 x. Specimen stained and mounted as in Fig. 1.

Fig. 7.—520 x. Specimen stained in aqueous solution of methyl-violet; mounted in aqueous solution of potassium acetate (1:5).

Fig. 8.—About 650 x. Specimen stained and mounted as in Fig. 7.

All specimens were hardened in 10% solution of formalin.

Outlines of figures were drawn with a Zeiss camera lucida.

136¹

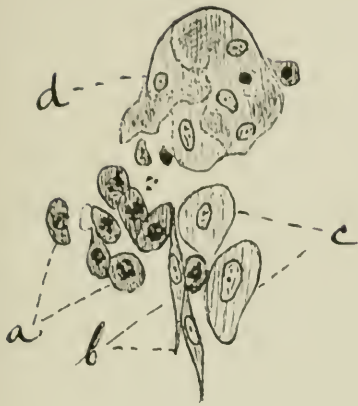


FIG. 1.

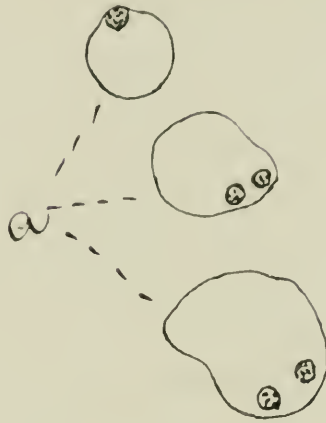


FIG. 2.

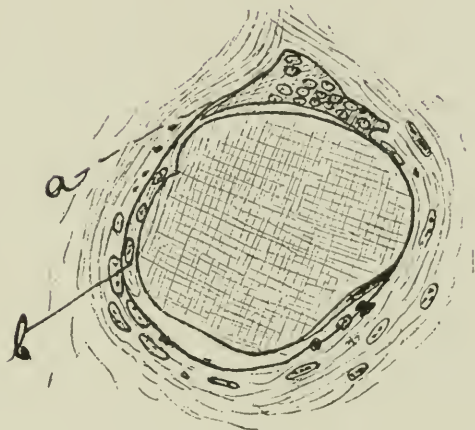
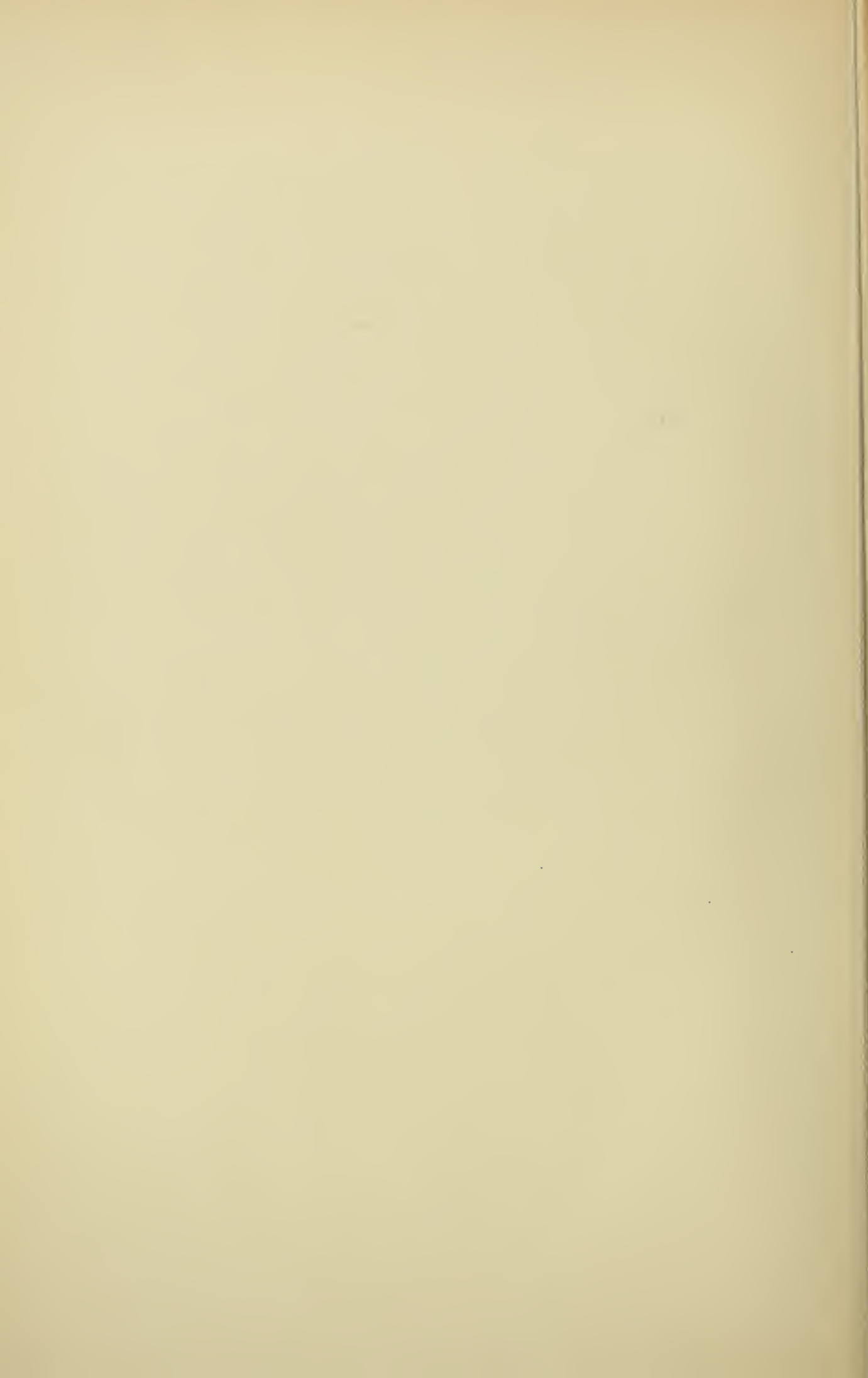


FIG. 3.



FIG. 4.



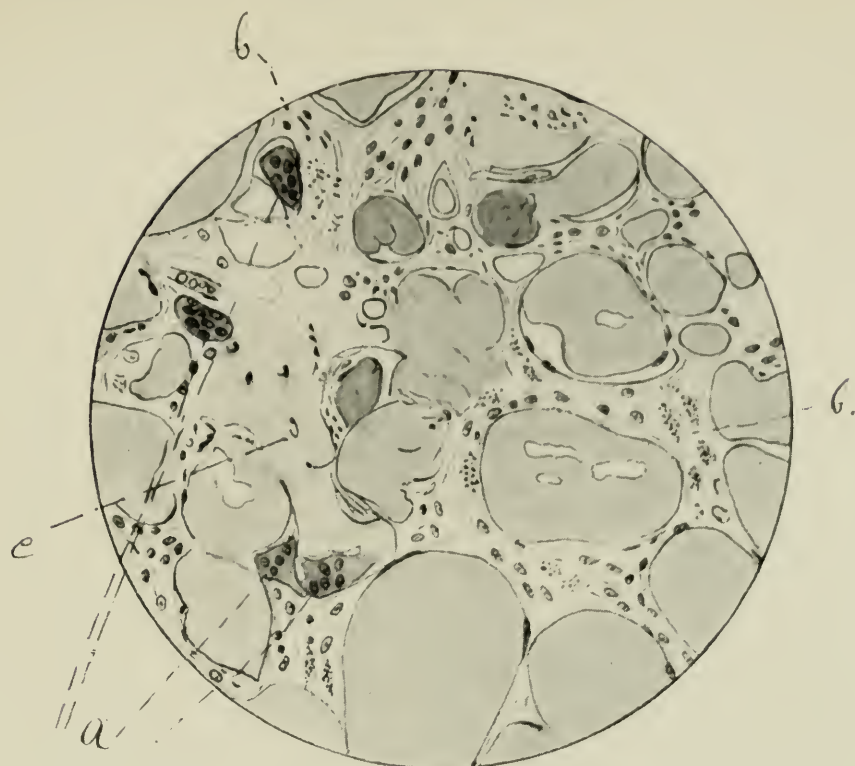


FIG. 5.

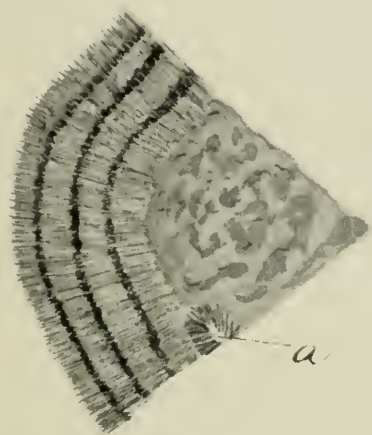


FIG. 7.



FIG. 8.

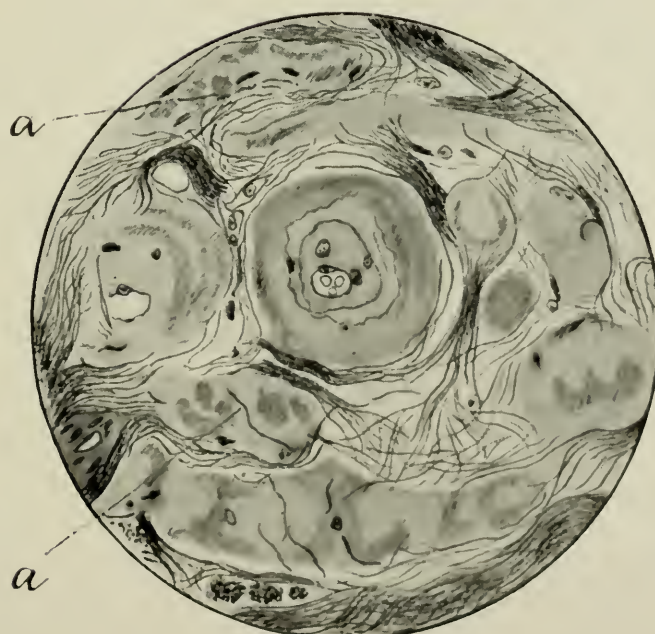
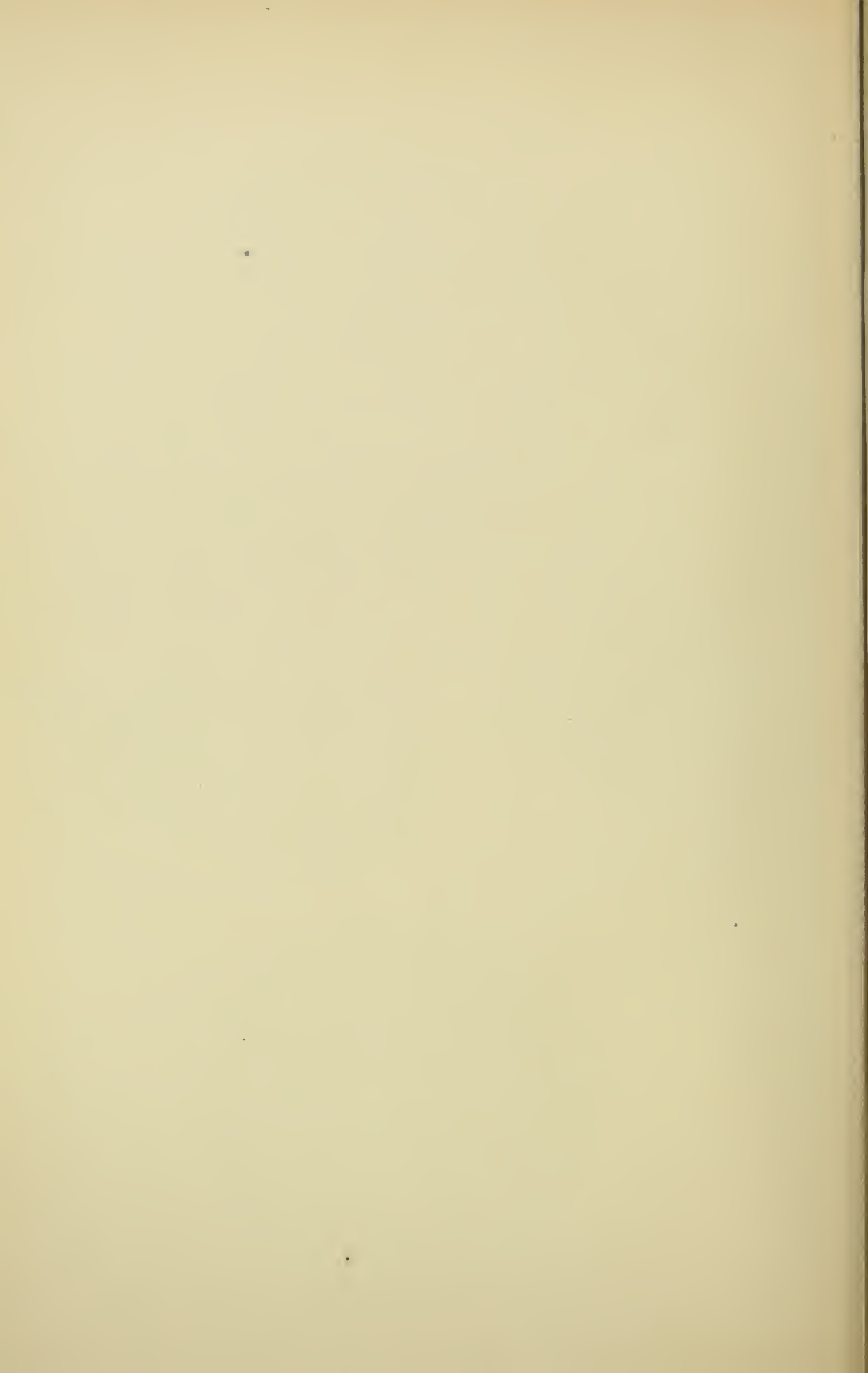


FIG. 6.



ON THE PRESENCE OF NEW ELASTIC FIBRES IN TUMORS.

BY ALICE HAMILTON, M. D.

(From the Pathological Laboratory of the Woman's Medical College of Northwestern
University, Chicago.)

PLATES VIII AND IX.

The question of the participation of elastic fibres in the hyperplasia of connective tissue has recently aroused a good deal of attention, especially since the discovery of differential stains has made possible the recognition of very delicate elastic fibres. Sclerotic blood-vessels seem to have been first and most extensively studied, with a view to ascertaining not only the fate of the elastic fibres in the media, but also the nature of the newly-formed fibres in the intima. Langhans¹ was the first to declare that this new tissue consisted largely of elastic fibres, and later Baumgarten² and Heubner³ confirmed this view. More recently Dmitrijeff⁴ and Jores,⁵ working with better staining methods, have described in detail the processes occurring in arteriosclerosis. Though they find minor differences in the syphilitic and non-syphilitic forms, the process in the main is found to be the same in all, namely, a granular disintegration and final absorption of the elastic fibres of the media followed by an extensive proliferation of elastic fibres in the intima. The thickened intima, in fact, consists almost wholly of delicate elastic fibres.

Both Jores and Dmitrijeff consider that the growth of these fibres in the intima is compensatory, to make up for the loss of elasticity in the media, but they differ in opinion as to the rapidity of their for-

¹ Virchow's *Archiv*, 1866, xxxvi, p. 201.

² Virchow's *Archiv*, 1878, lxxiii, p. 90.

³ Dieluetische Erkrankungen der Hirnarterien, Leipzig, 1874.

⁴ Ziegler's *Beiträge*, 1897, xxii, p. 207.

⁵ Ziegler's *Beiträge*, 1898, xxiv, p. 458.

mation. Dmitrijeff finds them always in the older parts of the tissue and thinks they are the result of a slow chronic process, while Jores finds them appearing in the very early stages of endarteritis proliferans. This opinion is confirmed by recent researches of Czyhlarz and Helbing.⁶ Jores believes that endarteritis proliferans should not be classed as a chronic inflammatory process, since it differs from such in any other tissue in this one important characteristic, the production of elastic fibres instead of white fibrous connective tissue. The hyperplastic connective tissue which results from chronic inflammation in other tissues, does not, according to his view, ever consist of elastic fibres. This assertion is disproved by the recent investigations of Melnikow-Raswedenkow.⁷ He seems to be the first who has systematically studied, by Weigert's staining method, the normal distribution of elastic fibres and the question as to their increase in different pathological processes. His investigations include the liver, spleen, lymphatic glands, heart, adrenal, and testicle. His conclusions are as follows: In hyperplastic connective tissue there is in almost all cases a proliferation of elastic fibres. This proliferation is always compensatory in character, either to make up for a loss of elasticity in the organ, or to increase its normal elasticity. Thus in cirrhosis of the liver the induration and loss of expansibility caused by the new connective tissue are not so great as they would be were the new tissue not composed largely of elastic fibres. In cicatricial contractions, especially in the myocardium, the new tissue contains many elastic fibres to diminish the evil effects of the contraction. In chronic passive congestion when the organ must force on a large volume of venous blood, we find the new tissue very rich in elastic fibres. On the other hand when there is no need for elasticity, the new tissue consists simply of the white fibrous variety, as in the thickened capsule of chronic perisplenitis and in the sclerotic patches in serous membranes. In almost all cases he finds these fibres derived from the adventitia of the blood-vessels; the fibres grow slowly and are very resistant to degenerative changes.

⁶ *Centralbl. f. path. Anat.*, 1897, viii, p. 849.

⁷ Ziegler's *Beiträge*, 1899, xxvi, p. 546.

The formation of elastic fibres being thus always compensatory, always for a definite purpose, Melnikow-Raswedenkow thinks there is no reason why they should share in the formation of new growths which are entirely harmful in character; and as a matter of fact, he finds that they do not play any part in such growths. He does not enter into the subject in detail, but states that he has formed this conclusion from the study of many cases of benign and malignant neoplasms; and mentions adenofibroma, fibromyoma, sarcoma, carcinoma simplex and scirrhus, and cystoma glandulare. He thinks that the frequent occurrence of degenerative changes in the centre of new growths is due to this lack of elastic fibres in their stroma and the consequent imperfect circulation.

In the discussion following Ziegler's presentation of Melnikow-Raswedenkow's investigations before the *Deutsche pathologische Gesellschaft*⁸ his results met general confirmation, but Hansemann stated that in a single instance (sarcoma of the lung) out of 150 malignant tumors examined he had found increase of elastic fibres, and Schmorl and Orth added that they had each found elastic fibres in a single tumor, the former in a gastric cancer, and the latter in a cancer of the thoracic duct.

I have been unable to find much that is definite on this subject in the writings of other authors⁹. It would seem at first sight entirely possible that, inasmuch as all forms of connective tissue are found as elements of tumors, this one variety should also be found, either as a minor part of the growth or forming its chief constituent. Also, considering that the stroma of tumors is formed from the stroma of the organ in which the new growth occurs, it would seem quite possible that when this organ is rich in elastic fibres the newly-

⁸ *Verhandl. d. deutschen patholog. Gesellschaft*, 2^{te} Tagung, p. 235, Berlin, 1900.

⁹ After this paper was completed and sent to the editor there have appeared the interesting articles of H. U. Williams, "Concerning the new formation of elastic fibres, especially in the stroma of carcinomata" (*Contributions to the Science of Medicine* dedicated by his pupils to William Henry Welch, p. 291, Baltimore, 1900), and of W. C. White, "The distribution of connective tissue in new growths" (*Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 185). These articles cannot be considered in this paper.

formed stroma should also contain them, as it contains new blood-vessels.

In investigating this question as to the participation of elastic fibres in the formation of neoplasms I chose the Weigert stain,¹⁰ which gives very excellent results, irrespective of the hardening fluid used. It can be used without counter stain, the blue-black fibres standing out against a light blue or almost colorless background; or, if it is desirable to stain the cells, the counter stain advised by Weigert, lithium-carmin, may be employed. A good contrast is obtained with hæmatoxylin and eosin, but a much more striking one by rapid treatment with Van Gieson's stain and hæmatoxylin. In such sections the cells are stained yellow, nuclei brownish, the white fibrous tissue pink and the elastic fibres blue-black.

As most investigators are agreed that the formation of elastic fibres in hyperplastic connective tissue is a slow process and that the new fibres come from the preexisting ones, I looked for the best results in benign connective-tissue growths occurring in tissues normally rich in such fibres, and therefore selected first for examination fibromata of the subcutaneous tissue. The result in most cases was disappointing. Elastic fibres were present in narrow strands between bundles of white fibres, but in such small numbers that it was impossible to be sure that they were not the preexisting fibres of the subcutaneous tissue which had been pushed apart by the new growth. In only one case were the fibres more numerous, irregularly scattered in thick twisted bundles. This same appearance was found in a myo-fibroma of the uterus. Better results were gained from adenofibroma and pericanalicular fibroma of the mammary gland where the proliferation of elastic fibres was often quite extensive. In the normal mammary gland elastic fibres of moderate thickness are found forming bands around the excretory ducts, while a few very delicate ones may be seen in the stroma between the lobules. In the above-named neoplasms these bands around the ducts are often increased in thickness and fibres can be seen running out into the surrounding tissue, while thick bundles of fibres are found in the interacinous stroma

¹⁰ *Centralbl. f. allg. Path. u. path. Anat.*, 1898, ix, p. 289.

as well as in the stroma between the lobules. The elastic tissue of the adventitia of the blood-vessels is increased and sometimes the fibres pass out into the surrounding tissue. In none of these cases, however, did the elastic fibres form a conspicuous element of the tissue, and in some they were very scanty in number. No tumor of this class was found which consisted of elastic fibres exclusively.

Turning then to the question of their presence in the stroma of malignant tumors I chose as the most promising the scirrhus carcinomata. The tumors examined were from the pancreas, mammary gland and liver. The distribution of elastic fibres in the normal pancreas is similar to that in the mammary gland, namely, in slender bands around the excretory ducts, a few delicate fibres in the interlobular tissue, and of course in the walls of the blood-vessels. The liver is much more abundantly supplied with these fibres, which are present in such large numbers in the interlobular connective tissue that they seem to leave little room for any other sort of tissue. Almost all of the scirrhus carcinomata from these organs which were examined for this purpose were found to contain large numbers of elastic fibres far more than had been found in any of the fibromata examined. The fibres in some cases seemed to come from the preexisting ones around the ducts or vessels, but were far more abundant than in normal tissue and passed from these regions to the surrounding stroma. This was especially true of the two cases of scirrhus of the liver, originating in the bile-duct epithelium. Not only the stroma of the new growth but the hyperplastic connective tissue throughout the organ consisted largely of elastic fibres which radiated from the adventitia of the blood-vessels and bile-ducts. In scirrhus of the pancreas the elastic fibres were not so numerous and were found in thick masses scattered irregularly in the stroma of white fibrous tissue. Two cases of scirrhus of the liver and one of the pancreas showed a peculiar arrangement of these fibres. They formed a series of quite well-marked concentric rings around a centre which was made up of carcinomatous cells in nests lying in a stroma quite free from elastic fibres (Plate VIII, Fig. 1). This appearance might be explained by the more rapid growth of this portion of the tumor, which had pushed

aside the elastic fibres of the more fully-developed stroma. Such an arrangement does not show at all in sections stained by the usual methods.

In scirrhus of the mammary gland the elastic fibres were found around the excretory ducts, around the nests of cancer cells and in the interlobular connective tissue. In some, these fibres were delicate, wavy, arranged in regular bands; in others, thick, short, twisted, arranged in heavy masses. Always the appearance was characteristic of elastic fibres—the variable thickness of the fibres, the wavy outline and curled ends. Usually they occurred in scattered groups, but occasionally they were woven in with the stroma quite evenly. In almost every case they formed a conspicuous element in the new growth.

Contrary to expectation, it was in the soft, malignant tumors of epithelial origin, where the connective-tissue stroma was delicate and there was every evidence of rapid development, that the richest growth of elastic fibres was found. In adeno-carcinoma of the uterus (Plate IX, Fig. 3), stomach and mammary gland (Plate VIII, Fig. 2), the stroma in many instances consisted largely of elastic fibres, and very seldom were they altogether wanting. In several cases these fibres were found only in the thicker trabeculae of the stroma, and in two instances the deeper part only of the tumor contained them, the fibres ceasing gradually as the margin of the growth was approached.

The only forms of sarcoma which promise success in such an examination are fibro-sarcoma and alveolar sarcoma, and good results were obtained in examples of both these groups. A melanotic alveolar sarcoma showed thick, twisted bands of elastic fibres in the stroma; a fibro-sarcoma showed delicate wavy fibres passing between the cells. In neither of these two was there any apparent connection between these fibres and those in the adventitia of the blood-vessels, but in a fibro-sarcoma of the brain this connection could be plainly seen. This tumor, which I have described in this Journal,¹¹ evidently originated in the outer walls of the blood-vessels and possessed a stroma made up entirely of elastic fibres, which ran in bands, radiating from the ves-

¹¹ *Journal of Experimental Medicine*, 1899, iv, p. 597.

sels, or were collected in rosettes. In this case all of the fibres were elastic, in the other two the background for these fibres was made up of slender bands of white fibrous tissue (Plate IX, Fig. 4).

It may be objected that the elastic fibres found in these tumors are simply those which were already present in the tissue in which the growth occurs. In answer to this, several arguments may be advanced to prove that there is in the cases described a formation of new fibres, which is sometimes very extensive. In the first place they are found in tumor masses where there is no question that we have to do with a newly-formed stroma, not an infiltration of the original tissues. In the second place they are found in numbers far greater than those normally present in the organ affected. Thirdly, they are found in scattered masses not in connection with vessels or ducts, although normally the organ in question contains elastic fibres only around the excretory ducts and blood-vessels. Thus in the scirrhus of the pancreas represented in Plate VIII, Fig. 1, the elastic fibres are abnormal in quantity and arrangement. Indeed the number found in the tumor often bears no relation to that normally present in the tissue in which the growth occurs. Several specimens of carcinoma of the uterus contained a stroma very rich in such fibres, while two examples of carcinoma of the lung and several of carcinoma in subcutaneous tissue contained none at all. In scirrhus of the liver different parts of the same tumor show different numbers of these fibres and their arrangement is very often, as in the pancreatic and mammary tumors, entirely different from that in the normal organs.

The term "proliferation" as applied to elastic tissue is, of course, rather loosely employed. Strictly speaking "formation" or "deposition" would be more accurate, though an exact term cannot be found until the nature of the process is better understood. The dispute between histologists as to the cellular or intercellular origin of these fibres is still maintained, and the same lack of agreement is found among those who have studied their formation under pathological conditions. Langhans and Dmitrijeff from their studies of arteriosclerosis conclude that the fibres originate in the intercellular matrix, while Heubner and Jores, working upon the same sort of tis-

sue, decide in favor of their cellular origin. It is impossible for me to speak decidedly on this point from my observations of new growths, but it is certainly true in my experience that the tumors containing the largest numbers of elastic fibres are those which possessed a stroma comparatively rich in connective-tissue cells, which would seem to indicate that the cells play an important part in the formation of these fibres.

DESCRIPTION OF PLATES VIII AND IX.

PLATE VIII.

Fig. 1.—Scirrhus carcinoma of the pancreas, showing elastic fibres arranged in rings around a central area. Weigert's stain—counterstained with lithium carmine. Leitz, Obj. 3; Oc. 3.

Fig. 2.—Alveolar carcinoma of the mammary gland. Weigert's stain—counterstained by Van Gieson's method. Leitz, Obj. 6; Oc. 3.

PLATE IX.

Fig. 3.—Alveolar carcinoma of the uterus. Weigert's stain—counterstained with lithium carmine. Leitz, Obj. 6; Oc. 3.

Fig. 4.—Fibro-sarcoma. Weigert's stain—counterstained by Van Gieson's method. Leitz, Obj. 3; Oc. 3.

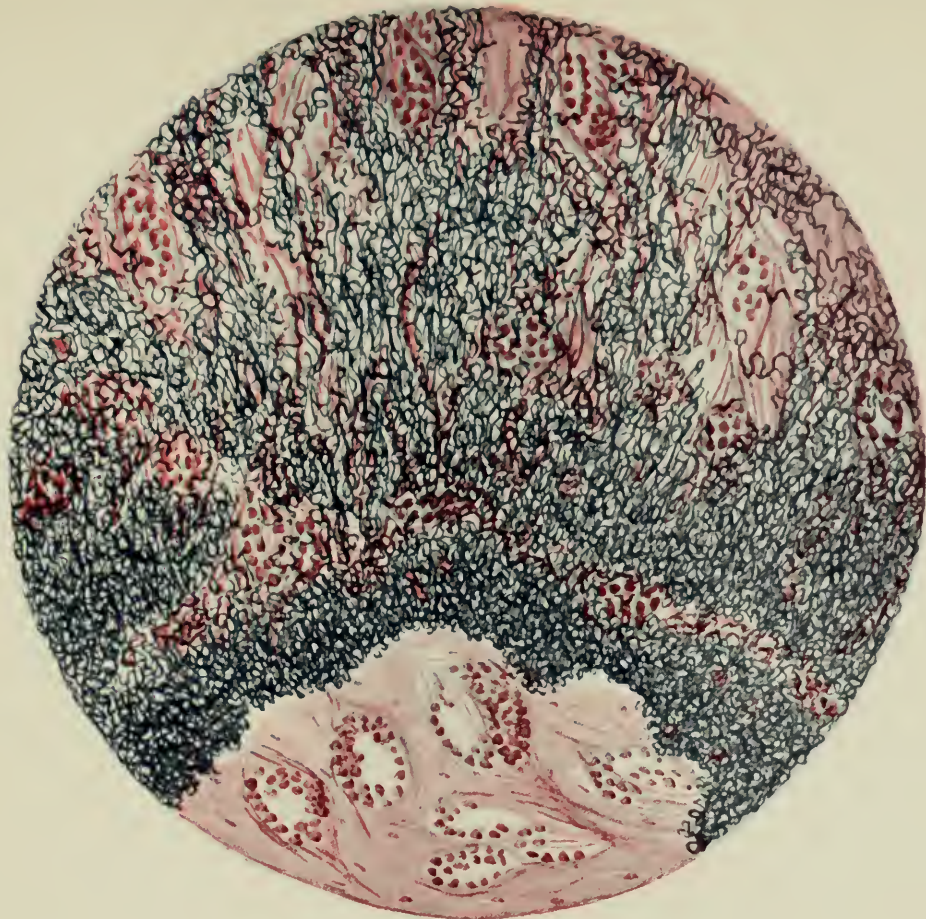


FIG. 1.

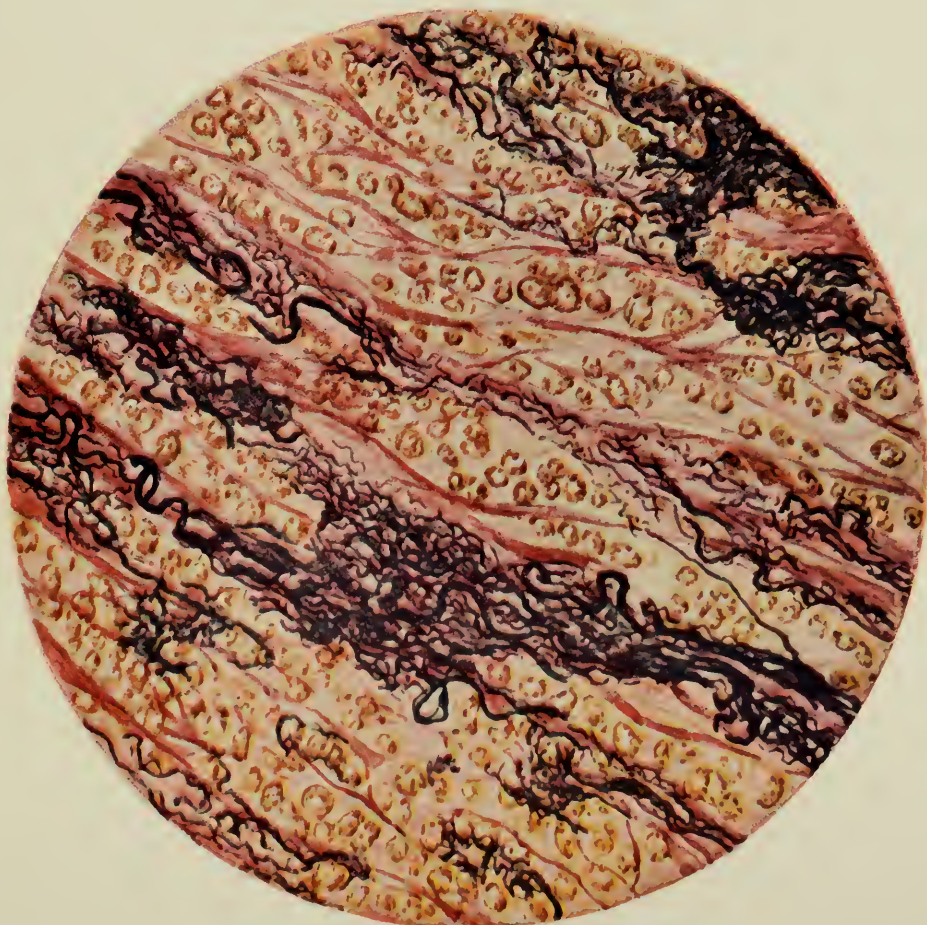
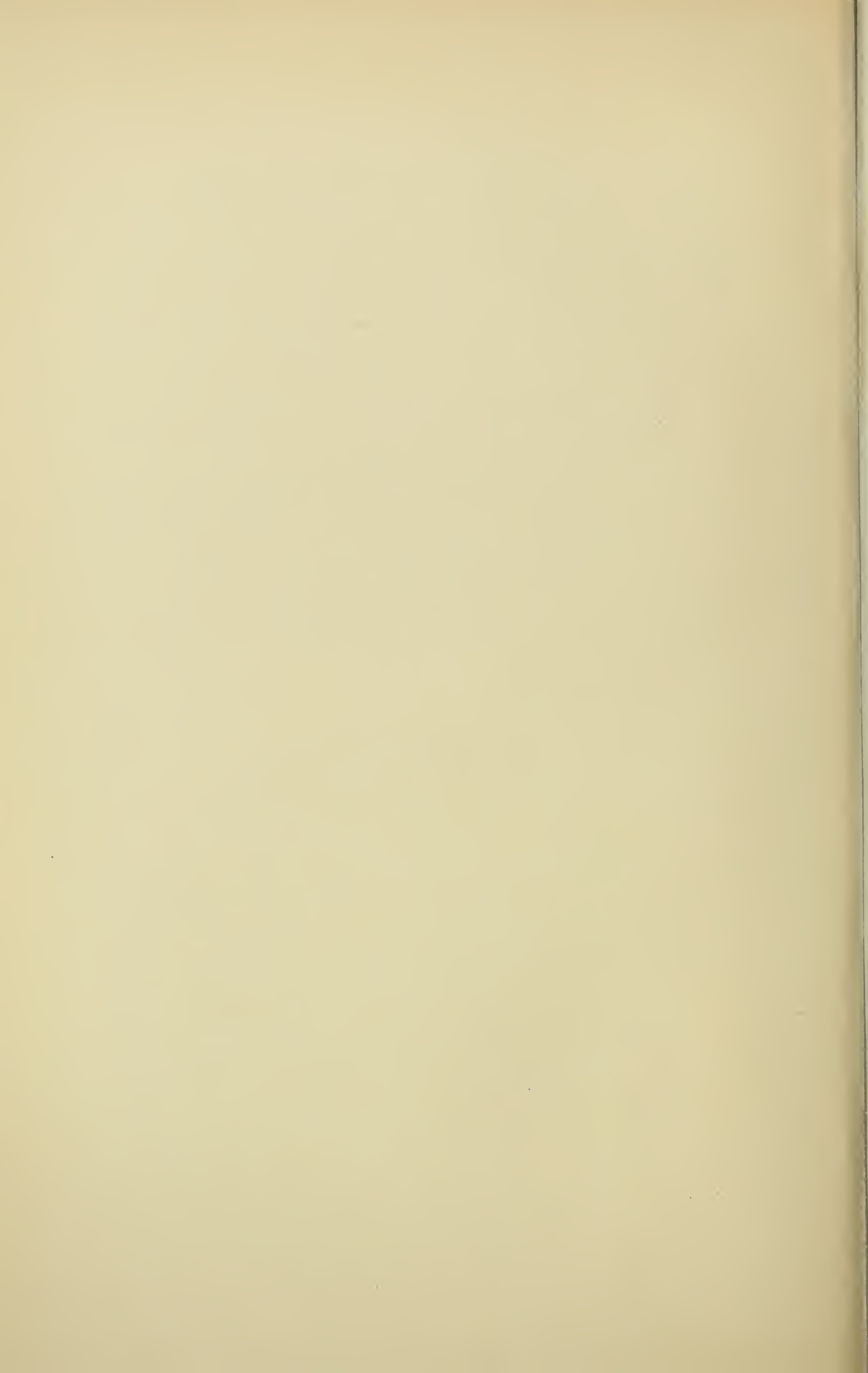


FIG. 2.



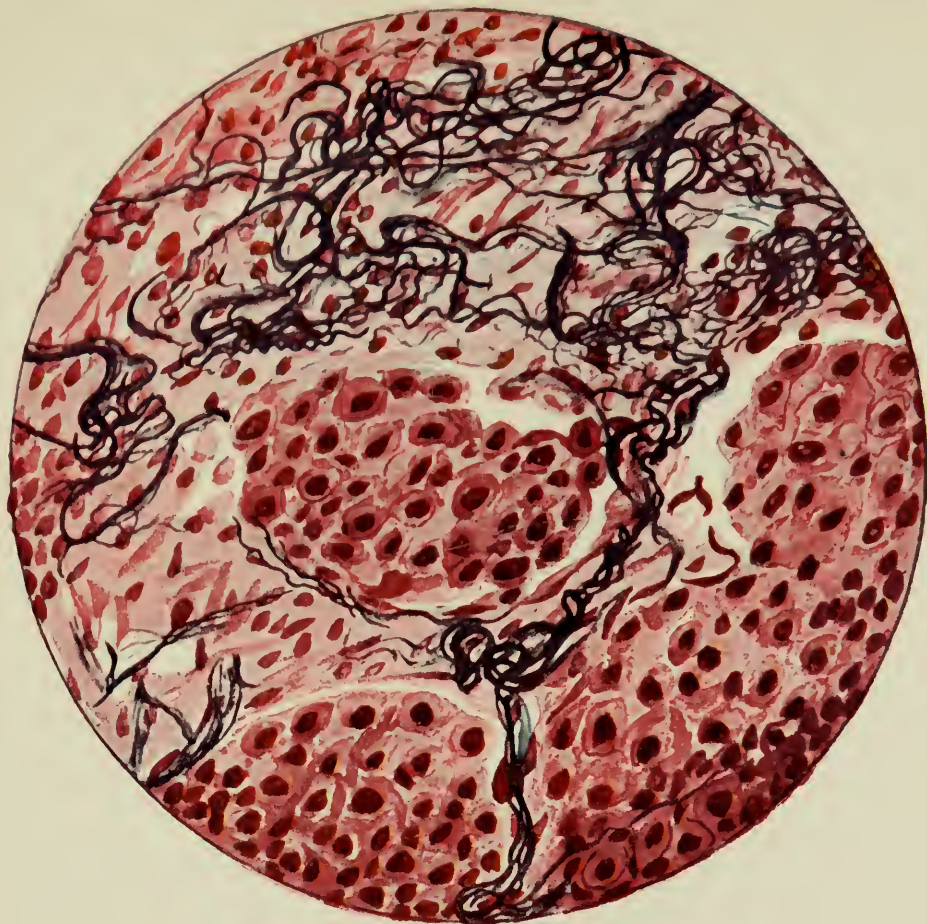
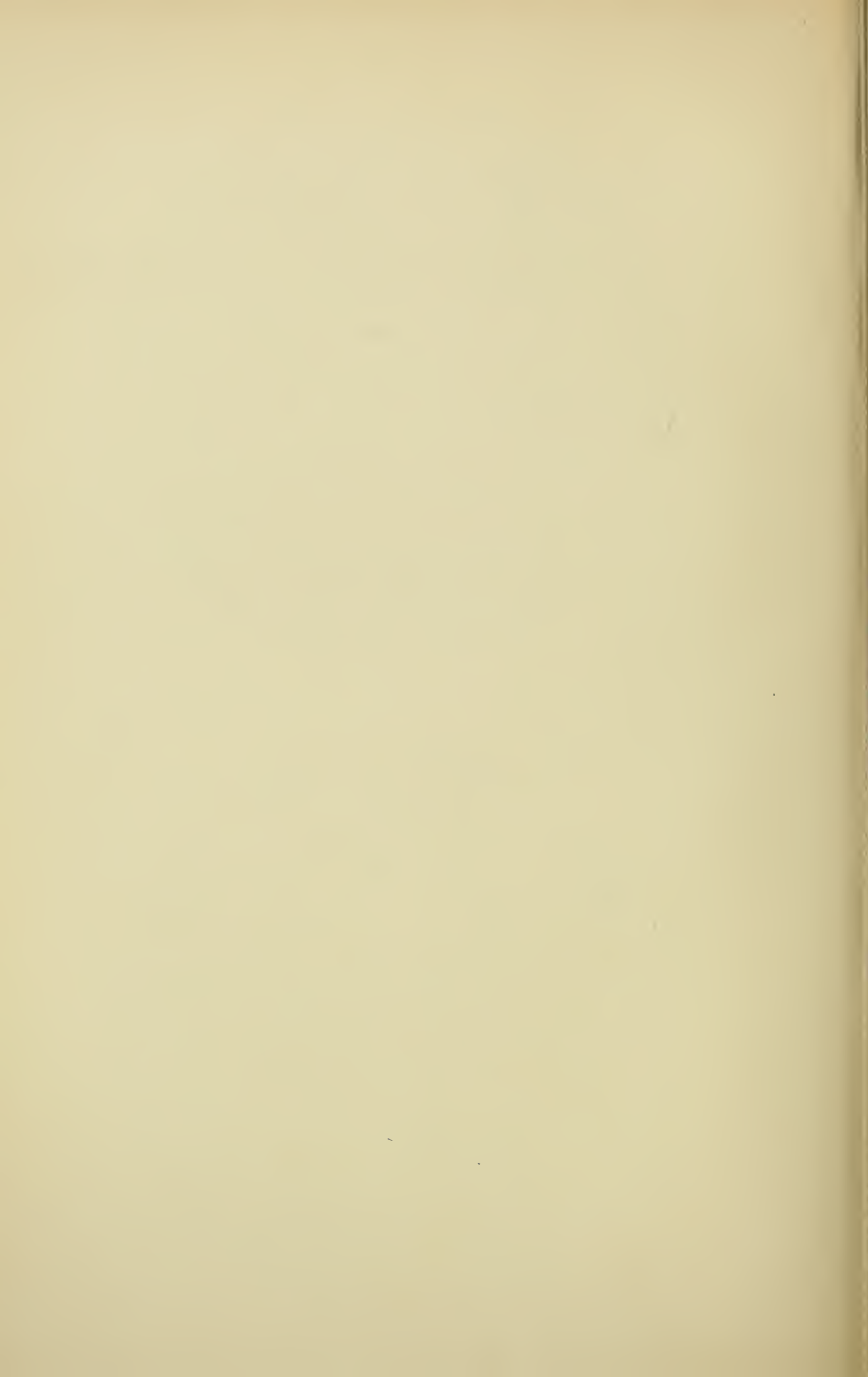


FIG. 3.



FIG. 4.



A CASE OF GENERAL GASEOUS EMPHYSEMA WITH GAS
CYSTS IN THE BRAIN FORMED AFTER DEATH AND
DUE TO BACILLUS MUCOSUS CAPSULATUS, WITH A
CONSIDERATION OF THE GAS-PRODUCING PROP-
ERTIES OF CERTAIN MEMBERS OF THIS GROUP IN
THE CADAVERS OF ANIMALS.

By W. T. HOWARD, JR., M. D.

(From the Pathological Laboratory of Lakeside Hospital, Cleveland, O.)

PLATE X.

My attention was directed to this subject by the following case:

M. D., female, white, aged 40 years, was admitted to Lakeside Hospital, service of Dr. H. S. Upson, December 24, 1898. On admission she was comatose and never regained consciousness. Her pulse was 108 to the minute, regular and rhythmic. Her respirations were of the Cheyne-Stokes type, apnoea lasting twenty seconds. The extremities were cold and cyanotic; the skin was cool and moist. Examination of the chest and abdomen showed nothing abnormal. There was incontinence of urine, and no urine could be obtained on catheterization; hence examination was impossible. Her friends stated that she was in the habit of taking large quantities of morphine. She died eight hours after admission. The clinical diagnosis was morphine poisoning.

No swelling or subcutaneous emphysema of the chest or other part of the body was noticed during life. Immediately after death the body was placed in the refrigerator, kept constantly at 30° F.

Autopsy.—Anatomical Diagnosis: General gaseous emphysema of the subcutaneous tissues, the heart, blood-vessels, liver, spleen and kidneys. Gas cysts of the brain. Septicæmia due to *B. mucosus capsulatus* (aërogenes group). Fatty degeneration and cloudy swelling of the heart, liver and kidneys. Chronic interstitial nephritis. Ulceration of the stomach and ileum.

The autopsy was begun 24 hours after death. The body was 157 cm. long, very cold; rigor mortis present. The skin over the posterior por-

tions of the body was of a deep purplish hue. The skin and subcutaneous tissues of the face, neck and chest were markedly swollen, and tympanitic on percussion. On section, the skin and subcutaneous tissues were crepitant to the touch and separated from the underlying structures. The subcutaneous and muscular tissues of the chest, neck and face were enormously distended with gas and marked emphysematous crackling was elicited on pressure. The circumference of the chest just above the mammæ was 108 cm. The head was of ordinary size, the scalp thick and firmly adherent. There were no wounds, contusions or abrasions about the face or head. The skull was of ordinary thickness. The dura mater showed no changes. All the sinuses contained dark fluid blood in which were a great number of large and small gas bubbles.

The meningeal vessels, both arteries and veins, contained a great number of gas bubbles. The veins were markedly congested. In the pia-arachnoid there were numerous small gas blebs. The cerebral convolutions were normal. The surfaces of the cerebrum and of the cerebellum showed no changes. There was no exudation, either serous or purulent, upon or in the pia-arachnoid. The structures at the base of the brain appeared normal.

On section the cerebrum was firm (frozen) and moderately congested throughout. The cortex cerebri appeared normal. In the internal capsule and in the lenticular nuclei, there were a large number of nearly round and oval gas cysts, varying from one to twelve millimetres in diameter. Near the centres of both cerebellar lobes, there were similar gas cysts varying from one to four millimetres in diameter. These cysts had perfectly smooth walls. The cerebellum was moderately congested. The pons and medulla both appeared normal on section. The arteries at the base were not thickened and appeared normal.

The spinal vertebræ were normal. Between the dura and the pia-arachnoid were a large number of large and small gas bubbles.

The extremities were free from œdema and emphysema. The abdomen was markedly swollen, measuring 105 cm. in circumference at the level of the umbilicus. The subcutaneous tissue was resonant on percussion, but no crackling was elicited. The peritoneal cavity and the intestines were distended with gas. The sternum, mediastinum and pleuræ were normal. Both lungs were voluminous, and showed small gas blebs under the pleuræ. On section the lungs were markedly congested, but crepitant throughout. Gas bubbles were expressed from the larger pulmonary blood vessels. The trachea, larynx, and mouth were normal. The pericardium was normal.

The heart weighed 240 grammes. The myocardium was pale and lustreless, and here and there gas cysts of varying size, surrounded by hyperæmic zones, were seen. All the cavities contained dark red fluid blood with gas bubbles. The valves and orifices were normal. The coronary arteries and veins contained small gas bubbles.

The diaphragm was of a pale yellowish hue and felt soapy to the touch, while on section small gas bubbles were seen.

The liver was of ordinary size. The capsule was for the most part smooth. At the under surface of the right lobe, near the gall-bladder, small gas bubbles were found just beneath the peritoneum. On section the liver was rather longer than usual, and had a pale yellowish-gray appearance, with a soapy sensation to the touch. All the visible blood-vessels contained gas bubbles, but the typical appearance of "Schaum-leber" was not present. The bile-ducts and gall-bladder were normal. The tissues about the gall-bladder contained small gas bubbles.

The spleen weighed 175 grammes. The capsule contained small gas bubbles. On section the organ was of a dark red color, soapy to the touch, and contained a large number of small gas bubbles.

The two kidneys were of the same size and presented the same general appearances. Together they weighed 325 grammes. The capsules were slightly adherent, the surfaces smooth. The cortices were pale and of ordinary thickness. Both the cortex and the medulla contained small gas cysts. Small gas bubbles were found in the pelves and in the veins. The adrenals and the pancreas were negative. The mucous membrane of the œsophagus was soft and easily removed with the finger. The stomach was of ordinary size. The mucous membrane was markedly congested. Near the cardiac end there were several small ulcers, with indurated bases and edges. Just above the ileo-cæcal valve three Peyer's patches were the seats of small ulcers presenting firm borders. The mucous membrane of the colon and rectum was congested.

The ovaries and tubes were embedded in a mass of adhesions, but presented no changes of special interest.

The bladder, ureters, lymph glands, and the arteries were negative.

The venæ cavæ, the jugular, axillary, portal, hepatic, splenic, renal, and other abdominal veins contained gas bubbles.

HISTOLOGICAL EXAMINATION OF HARDENED SECTIONS.—*Central nervous system*.—The veins and capillaries of the pia-arachnoid, the sulci and the cerebral cortex contained great numbers of polymorphic bacilli, which were in some places seen also in the mediæ and adventitia and in small numbers in the surrounding tissue. The bacilli in the vessels were

so numerous that the blood corpuscles were completely displaced. There was no inflammatory reaction. The ganglion cells stained well and there was no evidence of degeneration of the cortical tissue. Plate X, Figure 1 shows a cerebral vessel filled with bacilli.

The veins and capillaries of the pia-arachnoid over the pons, medulla and spinal cord contained great numbers of polymorphic bacilli. In the spinal meninges the vessels were dilated and crowded with bacilli. Some of the vessels were widely distended with gas and a varying number of bacilli were seen near the intima. In some places bacilli were found free in the tissues of the pia-arachnoid.

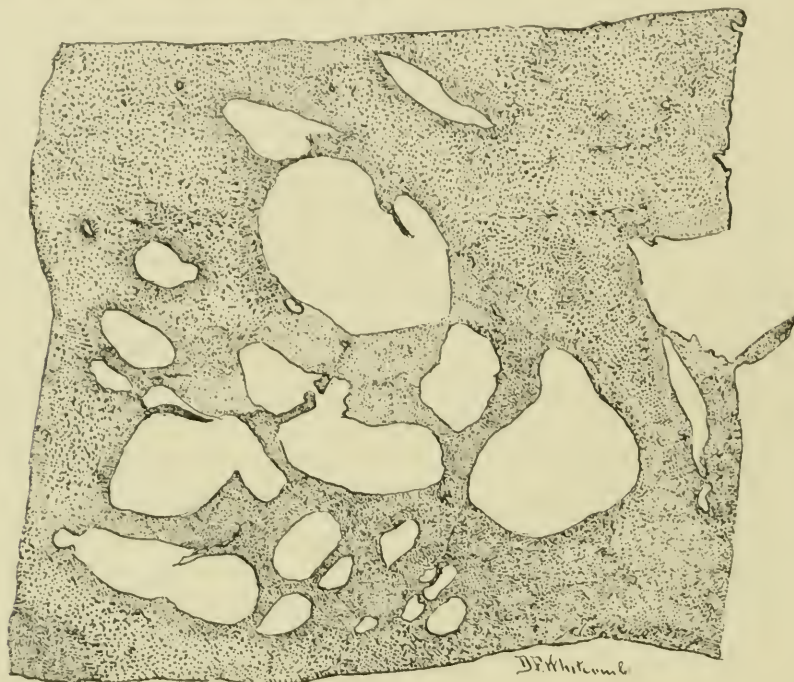


FIG. A.—Gas cysts in the brain.

Sections through the internal capsules and the lenticular nuclei showed large open spaces with regular walls (Fig. A). The tissues were pushed aside by the gas without any necrosis or liquefaction. The cells of the surrounding tissues stained well and appeared normal. There was entire absence of the cell degeneration about the gas cysts, described by Reuling and Herring¹ in their case of gas cysts of the brain. Along the walls of all the gas cysts polymorphic bacilli were seen in varying, and often in great numbers. In the neighborhood of the cysts, the veins and often the small arteries were filled with bacilli. Some of these vessels terminated in the gas cysts. Some, if not all of these cysts had their

¹ *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 62.

origin in dilatation or rupture of blood-vessels containing bacilli and gas.

In sections through the lateral ventricles the ependyma appeared normal.

The gas cysts in the cerebellum (Fig. B) were identical with those in the cerebrum.

In the internal capsules, lenticular nuclei, and cerebellum many of the veins and capillaries and some of the small arteries were crowded with small polymorphic bacilli. Some of the vessels of the cortex cerebri and of the pons and medulla contained bacilli. The spinal cord was free from gas cysts and its blood-vessels contained no bacilli. No degenerative changes were made out in the brain, pons, medulla, or cord. The ganglion cells were well preserved and stained well.



FIG. B.—Gas cysts in the cerebellum.

Heart.—In sections of the heart muscle the nuclei stained faintly and the cytoplasm took the eosin poorly. In some places the striation was indistinct and in others entirely lost. There were areas of marked segmentation of the muscle cells. In a number of small scattered areas the muscle tissue was broken up into a granular amorphous material. Many arteries, veins and capillaries were completely filled with polymorphic bacilli, which were also often found free in the tissues near the blood-vessels.

A large number of small smooth-walled gas cysts were found in the heart muscle. Along the margins of these cysts and in the neighboring tissues bacilli were present in large numbers.

Lungs.—Sections from both lungs showed marked œdema and congestion of the air vesicles, which often contained great numbers of polymorphic bacilli. The blood-vessels were congested, and contained a few bacilli. No gas cysts were seen.

Liver.—The interlobular connective tissue was increased in amount and in many places had encroached very much upon the liver lobules. In many of the interlobular spaces there was round-celled infiltration. There was no congestion of the blood-vessels. Fatty degeneration and cloudy swelling of the liver cells were marked and widespread. In many places the liver cells took the stain poorly, while in others they stained diffusely with eosin. The nuclei could not be made out. Some sections showed a number of gas holes, some of which were evidently due to dilatation of the central veins of lobules. The cysts varied very much in size.

Numerous bacilli were seen in the blood-vessels, especially in the intra-lobular capillaries and the branches of the hepatic vein. Many capillaries were widely dilated and contained no blood corpuscles but numbers of bacilli.

Spleen.—In the pulp there were few cells that were well preserved. The nuclei did not stain and the outlines of the cells were destroyed. Many cells did not take the eosin stain. The whole tissue had a peculiar amorphous appearance. A few of the Malpighian bodies were preserved. Here and there a few cells containing fat droplets were seen. The red blood corpuscles in the pulp and vessels were poorly preserved. There was no increase of polymorphonuclear leukocytes. No typical gas cysts were found in the spleen. Many of the blood-vessels contained myriads of polymorphic bacilli, the lumina of some being completely filled with them.

Kidneys.—The epithelial cells of the convoluted tubules were swollen and granular. The nuclei of many of the cells did not stain. The glomerular capillaries were congested. There was slight chronic interstitial nephritis, with well-marked chronic passive congestion. The large capsular veins and the arteries, veins and capillaries throughout the kidneys contained great numbers of polymorphic bacilli, the lumina of many of the vessels being completely filled with them. Bacilli were found in large numbers in the tissues, usually in the neighborhood of blood-vessels and within tubules. In many places the tubules were widely distended, forming gas cysts.

Stomach.—The superficial epithelium was lacking in many places. There was marked atrophy of the gastric tubules with round-celled infiltration of the mucosa. In sections through the ulcers only the deeper portions of the glands remained and sometimes both the glands and the muscularis mucosæ had disappeared. The submucosa was greatly thickened by a newly-formed granulation tissue rich in cells and blood-ves-

sels. The cells were fibroblasts, plasma cells and small round cells. A few polymorphonuclear leukocytes, but no eosinophilic cells were seen. No giant cells and no epithelioid cells were found. There was no special infiltration of cells about the blood-vessels and no caseation. Scattered through this granulation tissue, especially in the superficial portions, there were many polymorphic bacilli, quite similar to those found in other organs. No forms so large and thick as *Bacillus aërogenes capsulatus* were seen here or elsewhere in the organs. In some sections the veins of the submucosa were crowded with bacilli. The muscularis and serosa were normal.

Sections of the *small intestine* showed loss of the superficial epithelium with disintegration of the mucosa in places. In these areas the nuclei and cytoplasm did not stain and the cell outlines were lost. In this material there were numbers of bacilli similar to those found in the organs. The lymphoid tissue showed hyperplasia. In some places in the ileum the submucosa was infiltrated with plasma cells, but there was no granulation-tissue formation similar to that described in the stomach. No caseation and no giant cells were demonstrable. In one place near one of these areas the veins and capillaries were filled with bacilli.

Sections made from the subcutaneous fatty tissue of the neck showed a large number of larger and smaller round or oval gas spaces, the walls of which were lined with bacilli. There were no signs of inflammatory reaction. The tissues about these spaces were pushed aside and compressed. Bacilli were found in large numbers free in the tissue and in the small arteries, veins and capillaries.

Similar changes were found in sections of the pectoral muscles in which gas cysts were numerous. Many of the muscle cells had lost their striation, and in some the nuclei did not stain. Many bacilli were found in the tissues, blood-vessels and gas cysts.

BACTERIOLOGICAL EXAMINATION.—Smear preparations made at the time of the autopsy from the emphysematous subcutaneous tissue, the heart's blood, the lungs, liver, spleen, kidneys, cerebrospinal meninges, and brain, all showed great numbers of polymorphic bacilli, which occurred as almost round, oval, and short stout forms with rounded ends, and long thin filaments. They varied from 0.5 to 3 or even 5 μ in length, and were rarely more than 0.3 or 0.4 μ thick. Many were encapsulated. The capsules stained well by Welch's method. No thick bacilli resembling *B. aërogenes capsulatus* were seen, although careful search was made. Cultures were made from the subcutaneous tissue, the heart's blood, the liver, spleen, kidneys, lungs, brain, cerebral and spinal

meninges, upon blood-serum slants, and in glycerine-agar Petri plates. The cultures from each source, treated both aërobically and anaërobically, were kept in the incubator for twenty-four hours. Anaërobiosis was obtained by the use of Novy's jars.

In all the cultures, both blood-serum slants and Petri plates, the same microörganism grew abundantly and in pure culture. All the Petri plate cultures, both aërobic and anaërobic, showed a large number of round, raised, moist, smooth, opaque, greyish-white colonies. The deep colonies were small, irregular, finely granular, and of a deep brown color, when magnified fifty times. The superficial colonies were large (1 to 2 or 3 mm. in diameter), tended to spread, and microscopically showed irregular, greyish borders, with dark brown centres and a homogeneous appearance.

Cultures and coverslip preparations from all the plates showed the same organism, a polymorphic bacillus similar in all respects to those described in the fresh organs. No large thick bacilli were found in any of the cultures.

In the blood-serum slant cultures the colonies ran together forming a luxuriant spreading growth, in which it was difficult to separate individual colonies. The growth in these tubes was raised, moist, polished and porcelain-like. The water of condensation was cloudy. The growth stuck to the inoculating needle when it was removed from the culture, forming a ropy, mucus-like thread. Careful search failed to disclose any thick bacilli. Morphologically the bacilli in the blood-serum slants were identical with those found in the plate cultures and in the fresh organs.

Further study of this organism, found in pure culture in the subcutaneous tissues, brain, cerebrospinal meninges and other organs, gave the following results: Agar slants after 24 hours in the incubator, or 48 hours at room temperature, showed a luxuriant raised greyish-white, polished, porcelain-like growth, usually with serrated edges. Glycerine and glucose-agar slants gave the same growth. In both these media stab cultures showed abundant gas formation with splitting of the media. Gas bubbles were seen on the surface of the water of condensation, which was cloudy. On coagulated blood serum the growth was like that on agar; but here, in addition to appearing on the water of condensation, gas bubbles were also seen on the growth on the surface of the medium. Liquefaction of the medium did not occur.

On plate- and slant-cultures on gelatine the growth was similar to that on agar, though not so luxuriant. Gelatine was not liquefied. In stab cultures the growth occurred in the form of fine greyish-white colonies

along the track of the needle, with a flattened "nail-head" growth on the surface. Gas production did not occur in plain nutrient gelatine, but appeared in gelatine containing sugars.

On potato there was an abundant growth, best marked on the lower two thirds of the medium, where it was moist and of greyish-brown color. The upper third of the growth was usually dry and granular. The moist lower portion of the growth was porcelain-like and usually spread. Gas formation occurred in the growth, both on the medium and in the water about the potato.

In bouillon the growth was rapid and luxuriant, diffusely cloudy, with a white pellicle covering the surface. At the bottom of the tube there was a copious, greyish-white sediment, which, on shaking, was broken up into a stringy viscid mass.

In bouillon containing sugars abundant gas formation took place.

In Dunham's peptone solution there was a copious growth without indol formation. Blue litmus milk after 24 hours at body temperature had a pink tinge without coagulation. After 48 hours the medium was white with firm coagulation.

The organism was non-motile and stained readily and uniformly with the usual aniline stains. It decolorized slowly when treated by Gram's method. Spore formation did not occur. Capsule formation was sometimes seen in blood-serum cultures, and was constant in the blood and tissue juices of animals dying after inoculation with the bacillus.

Pathogenesis.—White mice died in 12 hours after inoculation with small doses of bouillon, blood-serum, or agar cultures either subcutaneously or intraperitoneally. The bacillus was found at the seat of inoculation, in the blood and in the various organs.

Guinea-pigs were killed within the same time by either subcutaneous or intraperitoneal inoculation. In the peritoneal cavity there was an excess of lymph containing peritoneal cells and leukocytes. Many of these cells enclosed bacilli, a large proportion of which were capsulated.

Rabbits succumbed to small doses (0.25 to 0.5 cc.) of a bouillon culture administered either intravenously or intraperitoneally. Capsulated bacilli were found in the blood and various organs. No gas formation took place in the body during life, but when the animal was kept in a warm place after death, gas was formed.

Rabbits inoculated intravenously with 0.5 cc. of a 24-hour old bouillon culture of the bacillus, killed five minutes afterwards and put in the incubator at body temperature, showed general subcutaneous gaseous emphysema, with gas cavities in the heart, blood-vessels, liver, spleen,

kidneys and free gas in the abdominal cavity with the bacillus in pure culture in the various organs. During the autopsy rabbits were inoculated intravenously with heart's blood, and with blood and tissue juice from the subcutaneous tissues of the chest, and killed. After remaining for 24 hours in a warm place (25 to 30° C.) they were found enormously swollen. At autopsy there was general gaseous emphysema of the subcutaneous tissues and typical "Schaumorgane." Careful examination of coverslip preparations failed to show the long and short thick forms of *B. aërogenes capsulatus*. There were, instead, great numbers of bacilli morphologically identical with those inoculated. Many capsulated forms were seen.

From the above it is seen that in this case there was septicæmia followed by general gaseous emphysema of the body, the latter developing in all probability after death, caused by an organism belonging to the group *B. mucosus capsulatus*.

I have compared the bacillus of this case with my *B. mucosus capsulatus* of hæmorrhagic septicæmia in man² and with three other similar bacilli obtained at autopsies during the past winter, and can find no differences, which would seem sufficient to warrant an attempt at the formation of a new class.

This bacillus is practically identical with my bacillus of hæmorrhagic septicæmia except that it gives up the stain more readily when treated by Gram's method. It is an active gas producer in sugar bouillon. When grown in one per cent glucose, saccharose, or lactose bouillon for 48 hours, gas displaces 60% of the medium in the upright arm of the fermentation tube in the case of glucose, 70% with saccharose, and 62% with lactose bouillon. It also produces a large amount of acid, the amount of normal NaOH solution required to neutralize 1 cc. of a 48-hour-old bouillon culture being for glucose bouillon cultures $\frac{1}{15}$ cc., for saccharose or lactose cultures $\frac{1}{25}$ cc.

The tabulated results of a comparative study of the gas and acid production of the bacillus of this case, of similar bacilli obtained at autopsies during the winter, and of my bacillus of hæmorrhagic septicæmia in human beings are given below. For convenience the bacilli

² *Journal of Experimental Medicine*, 1899, iv, p. 149.

will be styled No. 34 (the bacillus of the present case), No. 45, No. 46, No. 68 (the numbers of the autopsies from which they were obtained), and H. S. (my bacillus of hæmorrhagic septicæmia in man). Autopsy No. 45 was a case of general arteriosclerosis, with thrombosis of the pulmonary artery and pulmonary infarctions, with *B. mucosus capsulatus* in the lungs. No. 46 was a case of chronic ulcerative colitis and proctitis with abscess of the left thigh, and a fistulous track communicating with the colon; thrombosis of the pulmonary artery and purulent bronchitis. *B. coli communis* was obtained in cultures from the colon, the abscess of the thigh, and the heart's blood, liver, and spleen, while *B. mucosus capsulatus* grew in pure culture from the lungs. Autopsy No. 68 was a case of sarcoma of the right frontal lobe of the cerebrum, and acute croupous pneumonia. *B. mucosus capsulatus* was obtained in pure culture from the affected lung and the pleura, and also from the uterus. Bacillus H. S. was obtained from the heart's blood and organs of a case of hæmorrhagic septicæmia.³ In none of these cases, except No. 34 (the present case), was there gaseous emphysema.

TABLE OF COMPARATIVE GAS PRODUCTION IN SUGAR BOUILLON.

Bacillus.	1% glucose bouillon.	1% saccharose bouillon.	1% lactose bouillon.	
No. 34	60%	70%	62%	The per cent refers to the ratio of liquid displaced by gas in the upright arm of the fermentation tube.
H.S.	65%	73%	50%	
No. 45	60%	75%	80%	
No. 68	30%	70%	61%	

TABLE OF COMPARATIVE ACID PRODUCTION IN SUGAR BOUILLON.

Bacillus.	1% glucose bouillon.	1% saccharose bouillon.	1% lactose bouillon.	
No. 34	1/13	1/20	1/20	Fractions represent amount, expressed in cc., of normal NaOH solution required to neutralize 1 cc. of bouillon culture.
H. S.	1/13	1/25	1/15	
No. 45	1/30	1/25	1/15	
No. 68	1/25	1/30	1/25	
No. 46	1/30	1/30	1/20	

Strong⁴ after an elaborate study of various capsulated bacilli, based more especially upon their gas and acid production in glucose, saccharose and lactose bouillons, divides them into two groups: (1) the Friedländer group, comprising *B. pneumoniae* Friedländer, *B. ozænæ* Fasching, *B. sputigenus* crassus, Bacillus Wright and Mallory, and

³ See reference No. 2.

⁴ A Study of the Encapsulated Bacilli, *Journal of the Boston Society of the Medical Sciences*, 1899, iii, p. 185.

possibly *B. rhinoscleromatis*, in which gas-production is most abundant with saccharose, slightly less with glucose, scanty or entirely absent with lactose; slight or no acid formation with lactose; and no coagulation of milk; and (2) the *aërogenes* group with "more abundant and constant gas formation on all three media; rapid coagulation of milk; and equal amounts of acid formation on all three sugars."

A glance at the tables shows that all four of our bacilli belong to the latter group.

In order to test the relative gas-producing properties of these bacilli in the animal body, rabbits were inoculated intravenously with 48-hour old glucose bouillon cultures of each bacillus. All the rabbits died within 24 hours, without any development of gaseous emphysema during life.

A second group of rabbits were inoculated intravenously, each with 1 cc. of a 24-hour old glucose bouillon culture, which was followed in a few minutes by an intravenous inoculation of 2 cc. of a 20 per cent solution of glucose in sterile distilled water. The rabbits were killed five minutes later and put in the incubator for eight hours, after which time there had developed a slight subcutaneous emphysema, most marked in rabbits inoculated with bacillus 34, and bacillus H. S. The rabbits were all kept sixteen hours longer in a hood at 28° C., when they were found very much swollen. At autopsy they all showed very much the same changes. The subcutaneous tissues of the chest, neck and axillæ were ballooned up and gave distinct emphysematous crackling on pressure. There was usually slight emphysema of the tissues of the abdominal wall and of the thighs. The abdomen was distended with gas. In all the animals the liver was dark greyish-brown in color, soft, and very friable, with emphysematous crackling on pressure, and a peculiar soapy sensation to the touch. The livers were disintegrating. The heart contained dark red blood with a few gas bubbles. Small gas bubbles were sometimes seen in the myocardium. The lungs were distended with gas and well preserved. The spleen showed no special changes. The kidneys showed a few gas cavities, and on pressure gas bubbles escaped from the blood-vessels. In the kidneys of the H. S. rabbit the gas cavities were especially well marked (Plate X, Fig. 2).

A third set of rabbits received intravenous inoculations of 1 cc. of one per cent lactose bouillon cultures of bacilli and a few minutes later 1 cc. of a 10 per cent solution of lactose in sterile distilled water. These

animals were killed five minutes afterwards and treated in the same manner as the second set. At the end of 24 hours they showed subcutaneous gaseous emphysema, and the same visceral changes described for the second set.

The first set of animals (those killed in twenty-four hours by intravenous inoculation of glucose bouillon cultures of the bacilli, but receiving no sugar injection) after remaining in a hood kept at 28° C. for twenty-four hours were very much swollen. The subcutaneous tissues contained considerable gas. Gas was also present in the peritoneal cavity, in the heart and blood-vessels, liver and kidneys. The liver had the same appearances as described for the rabbits inoculated with glucose and lactose. In these animals the gas formation was distinct, but not so abundant as in animals receiving glucose or lactose solutions before death. In several rabbits the stomach wall had ruptured.

In the animals receiving lactose the gas formation was most marked and in these there was a strong odor of putrefaction. The muscles of the chest and thigh were beginning to soften, and on section a thin dark brown juice escaped. Two control rabbits killed at the time of the above experiments and kept under the same conditions for thirty-six hours showed no gas production.

At the autopsies on these animals the bacilli inoculated were recovered in pure culture from the subcutaneous tissues and the heart, lungs, liver, kidneys, in both coverslip preparations and in cultures, both aërobic and anaërobic cultures being made. As a further precaution coverslip preparations were stained by Gram's method. The organisms obtained on the coverslips decolorized completely by Gram, with the exception of the bacillus H. S. By these precautions *B. aërogenes capsulatus* was excluded as a post-mortem invader in these experiments.

HISTOLOGICAL EXAMINATION.—Portions of the heart, lungs, liver, spleen, kidneys, muscle, and subcutaneous tissues of the axillæ from each of the various rabbits were hardened in formalin, sectioned and stained with eosin and methylene blue. Very much the same changes were found in the various organs.

The muscle fibres of the heart stained diffusely with eosin; the striæ were lost, and there was segmentation of many of the muscle cells. Only a few nuclei of muscle cells took the blue stain. The cells of the vascular walls usually stained. Large numbers of bacilli were seen in every field of the microscope. They were usually in capillaries and small veins, but were sometimes found free in the tissues. In the sections from the lactose rabbit, a few small gas cysts were found. The lungs

were well preserved and the cells stained well; the blood-vessels and many of the alveoli contained great numbers of bacilli. Some of the alveoli were distended to five or six times their ordinary size and about their margins bacilli were found. The livers showed most marked changes. In most sections the outlines of the liver lobules were entirely lost and the capillary walls had disappeared. There were very few well preserved liver cells in any of the sections. The liver cells were fused together into rows or columns of homogeneous material, in which no nuclei and no cell structure could be made out. These rows and columns were separated by small spaces, corresponding to the capillary spaces. In these large numbers of bacilli were seen. There were no well-defined gas cysts, but the tissue contained a number of irregular spaces. In some sections, especially in those from 46 (lactose), the portal veins were well preserved, and contained red blood cells and bacilli.

The kidneys were fairly well preserved and the nuclei and cell bodies stained well. In all the kidneys, however, in scattered areas, the nuclei of the epithelial cells of the tubules and the cells of the glomeruli refused the stain, while the cytoplasm stained diffusely with eosin and had a granular, coagulated appearance, resembling that of coagulative necrosis. In some places, however, the cells had fused together and presented a homogeneous appearance. In the areas of cellular change the capillaries contained large numbers of bacilli. Most of the veins and nearly all the capillaries contained bacilli in varying numbers. In some sections, as was especially well shown in the kidney of the rabbit inoculated with bacillus H. S. and lactose solution, well marked gas cysts from a pin's point to one or two millimetres in diameter were found. The tissues about these cysts were pushed aside and compressed and large numbers of bacilli were found along their walls. The subcutaneous tissue and muscle collapsed on removal from the body, so that no gas cysts were found in sections made from them. These tissues, however, contained great numbers of bacilli. The bacilli found in the various organs of these animals were apparently of the same species. They varied very much in length, from short oval, almost round forms to threads 6 to 8 μ in length. The long forms were thinner than *B. aërogenes capsulatus*, and usually had square ends.

Reuling and Herring⁵ have reported a case of cavities in the brain produced by *B. aërogenes capsulatus*. The identification of

⁵ *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 62.

this bacillus in their case depended upon the morphological appearances of the organism in the hardened sections and upon the lesions in the tissues. The cysts were not discovered until after the brain had been hardened in formalin; hence cultures and animal experiments were not made.

In the case of gas cysts with abscess of the brain and cerebrospinal meningitis, and general gaseous emphysema, reported by me,⁶ the causal relation of *Bacillus aërogenes capsulatus* was established by coverslip preparations, cultures, animal experiments and sections of the hardened tissues.

In view of the present case one cannot agree with Reuling and Herring in concluding that *B. aërogenes capsulatus* was necessarily the cause of the lesions in the two cases of "holes in the brain" with cysts in the heart, lungs, liver, and kidneys, described by Savage and White⁷ as cases of "universal cystic degeneration." In the light of our present knowledge, however, it is almost certain that the cysts in these cases were due to the agency of gas-producing bacteria, and probably, on account of its common occurrence in this rôle, to *Bacillus aërogenes capsulatus*.

SUMMARY.

In the case reported in this article there was septicæmia with special localization of the microorganisms in the brain, with gas cysts of the brain and general gaseous emphysema due to *Bacillus mucosus capsulatus* (aërogenes group).

The bacillus isolated from the organs of this case, as well as other members of the aërogenic group of *Bacillus mucosus capsulatus* can cause general gaseous emphysema in the cadavers of rabbits, either with or without the intravenous injection of sugar before the animal is killed, the gas, however, being most abundant and rapidly formed in the former case.

It is not impossible that some of the published cases of gaseous emphysema in which a bacteriological examination was not made,

⁶ *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 66.

⁷ *Trans. Path. Soc. London*, 1883, xxxiv, p. 1.

may have been due to members of the *Bacillus mucosus capsulatus* group.

Dr. Welch has called my attention to the possibility that diabetes may have existed in the case reported in this article. As no examination of the urine could be made, this possibility must be admitted. There is evidence that certain bacteria incapable of producing gas in tissues and organs of the body under other conditions may do so in diabetics on account of the presence of an abundance of sugar.

DESCRIPTION OF PLATE X.

Fig. 1.—Photograph showing *B. mucosus capsulatus* in a cerebral blood-vessel.

Fig. 2.—Photograph showing gas-cysts and masses of bacteria (*B. mucosus capsulatus*) in rabbit's kidney.

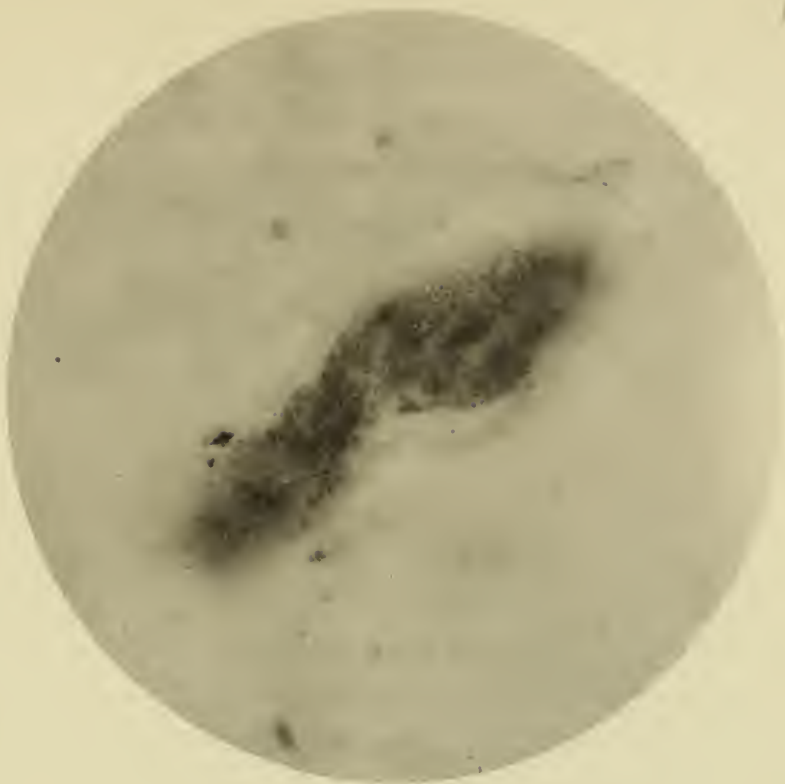


FIG. 1.

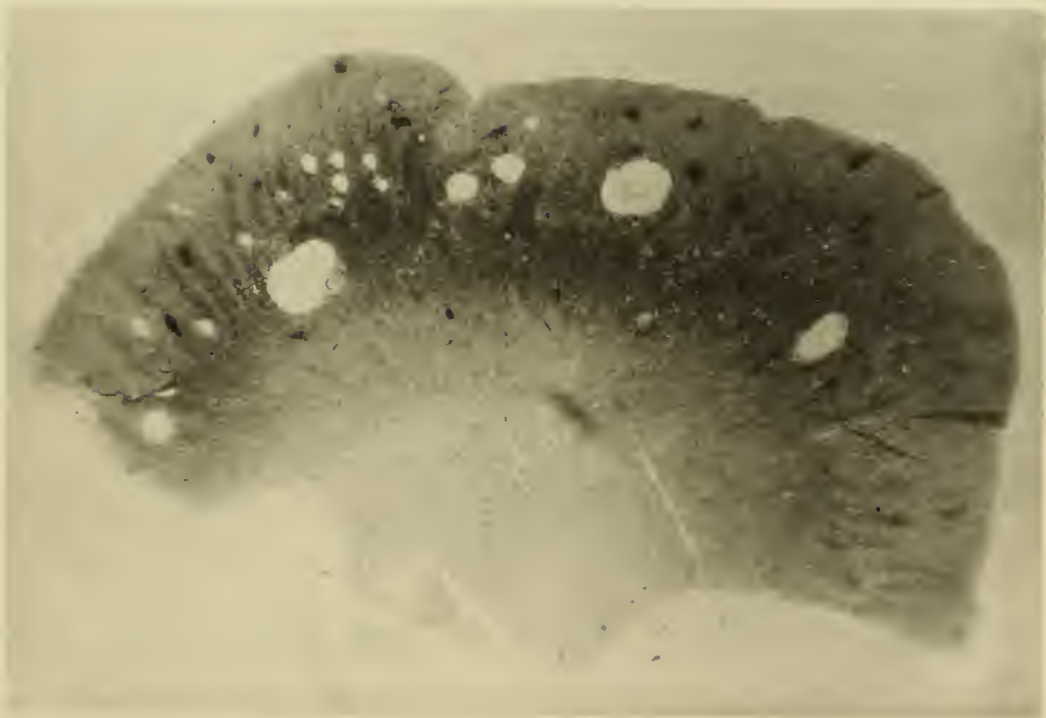
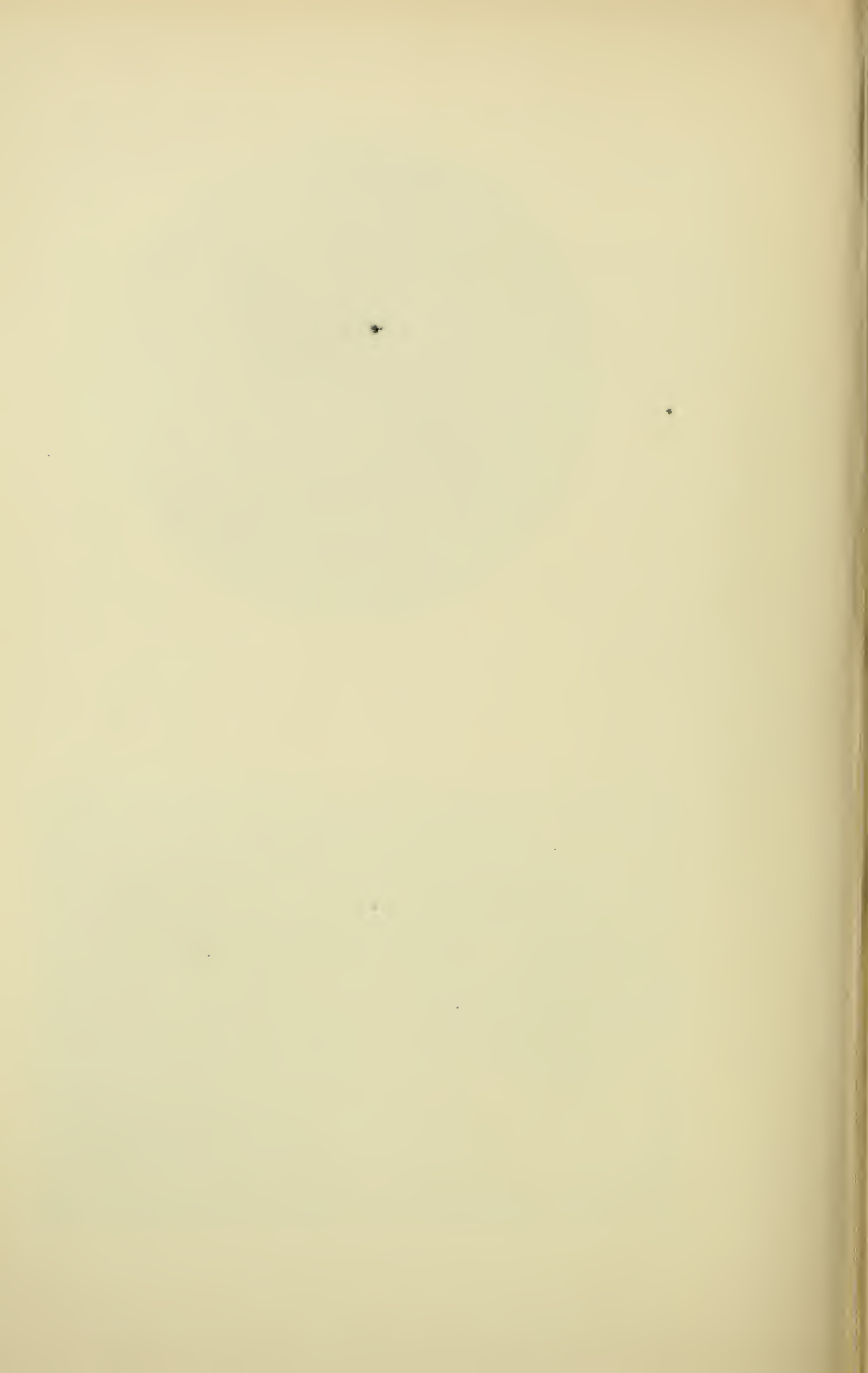


FIG. 2.



TWO CASES OF NECROTIC BRONCHO-PNEUMONIA WITH STREPTOTHRIX.

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PLATES XI-XVI.

The rarity and importance of such cases as form the basis of this paper, the inadequacy of the study of the similar cases already recorded, and the interesting, though as yet obscure, relationship between an organism or group of organisms which have long been known as actinomyces and the more recently-named streptothricaceæ, have urged us to the somewhat extended study of our material, which it is the purpose of this paper to record.¹

While the clinical histories are incomplete, the lesions, especially of the lungs, are well defined, and the characters and relationships of the microorganisms present seem to us to be of considerable general as well as special and technical interest.

CASE I. *Clinical History*.—J. L., aged 45, carpenter, was admitted to St. Francis Hospital, December 15, 1898. No history of previous illness. For the past six weeks patient had suffered from cough and moderate dyspnœa. Breath was extremely fetid. Patient was fairly well nourished.

Physical examination on admission revealed a nearly complete consolidation of the right lung, most marked in the lower lobe. Some consolidation of the left lower lobe. The temperature was always high, ranging from 103 to 104° F. The patient did badly and, on account of his continuous coughing and the foul odor of his breath and expectoration, he was isolated.

The clinical diagnosis was gangrene of the lung, following lobar pneumonia. He grew rapidly worse and died eight days after admission.

¹ A brief preliminary report of these cases was made to the New York Pathological Society on March 8, 1899.

Autopsy (Dr. Larkin), two hours after death, December 23, 1898. Body well developed and muscular; no rigor mortis or post-mortem lividity. The abdominal cavity contained a slight amount of straw-colored serum. The left lung was bound down by old adhesions; the lymph nodes at root of lung were very large, measuring from two and a half to three inches in length and one inch in breadth. The mucous membrane of the trachea and bronchi was cedematous, intensely congested, and bestrewn here and there with whitish masses resembling actinomyces "granules." The right lung was bound down by old adhesions and entirely consolidated. Bronchiectatic dilatations were present throughout the lungs. The cut section of the lungs, which were markedly anthracotic, was smooth, glistening, and mostly dark bottle-green in color. The consolidation was firm and resembled that of an organizing pneumonia. The connective tissue septa of the lung were cedematous and grayish in color. Myriads of small yellowish-white foci were scattered through the lungs. The odor of the lungs was extremely fetid.

The kidneys, liver, spleen, pancreas, intestine, bladder, and prostate appeared normal.

HISTOLOGICAL EXAMINATION.—The microscopic study embraced only the lungs, which were preserved in alcohol. The sections of the various lobes exhibit striking similarity in their microscopic appearances. The anthracosis is not so extensive as we were led to expect from the color and gross appearances of the lungs. The pigment is present in moderate amount in the peribronchial and pleural tissues.

Bronchi.—In places there is a simple catarrhal bronchitis with desquamation of the epithelium, fibrin and leukocytes. The coats of the bronchi are congested and infiltrated with serum and pus cells. The surrounding alveoli are filled with pus cells and a little fibrin. The simple catarrhal bronchitis and peribronchitis are confined to a few tubes. Besides the filling up of the terminal bronchioles and alveoli with leukocytes, a few small miliary abscesses or groups of alveoli filled with leukocytes are found. In the exudate of such alveoli and bronchi only a few streptococci and filaments or rods are present.

Many of the bronchi contain the filamentous colonies already mentioned as suggesting actinomyces granules at the autopsy. Where these are present the bronchi are the seat of a severe and destructive lesion, a necrotic suppurative bronchitis with dilatations, and here the bronchial structure is represented by a tissue staining deeply with eosin, composed of cells with ill-defined outlines and with fragmented nuclei. The

intimate relation of the bacterial or filamentous colonies to the necrosis is clearly seen in the places where the necrosis is strictly limited to the site of attachment of the colonies, although usually the whole circumference of the bronchus is implicated (Plate XVI). The necrosis may affect only the mucosa, or extend through the bronchial coats and about the bronchial dilatations, which contain the largest bacterial colonies, also to the adjacent, compressed and distorted zone of alveoli, which are filled with a coarse network of fibrin staining deeply with eosin. The leukocytes in the lumen of the bronchi near these colonies are in all stages of necrosis and degeneration, and the large amount of detritus found in the tubes is composed mainly of broken-down pus cells.

Pulmonary parenchyma.—The broncho-pneumonic lesion presents two phases of inflammation, which vary in importance and extent in different places. There are, first, zones of peribronchial alveoli filled with leukocytes in a marked condition of nuclear fragmentation, and with fibrin and epithelial cells. The alveolar epithelium in such regions shows little tendency to proliferation, and the alveolar walls are thickened by capillary congestion, by a cellular exudate composed of leukocytes and by epithelioid cells or fibroblasts. Adjacent to or surrounding such alveoli, are second areas of inter- and intra-alveolar pneumonia. Here the alveolar walls are much thickened by a proliferation of polyhedral or epithelioid cells and the fibrin in the alveoli is in all stages of organization. Vascularized plugs of connective tissue obliterate the alveoli here and there. In some sections the areas of interstitial pneumonia are quite extensive. These areas may directly touch upon bronchi which are the seat of an intense necrotic inflammation. Nuclear fragmentation and slight necrosis of the new tissue are observed even at some distance from the bronchus.

The adventitial coats of the arteries, and the connective tissue surrounding the larger bronchi and vessels are much thickened by a serous exudate. In places the necrosis has extended to the walls of the blood-vessels, and the alveoli are filled with red corpuscles. In some sections the hæmorrhagic areas are quite extensive.

Besides the extreme congestion of the capillaries and blood-vessels, few vascular changes exist. Some of the smaller arteries present an obliterating endarteritis. In the larger vessels considerable fibrin and a homogeneous material resembling coral-thrombi are seen. Cocci and filaments are not found in the blood-vessels. The lymph spaces of the bronchi and interlobular septa contain a few streptococci.

The pleura is covered in places by a layer of fibrin and leukocytes,

and is congested, thickened and infiltrated by a serous exudate and by a growth of new tissue. Streptococci are present in the fibrin and in the pleural lymph spaces. Extending from the pleura the interlobular septa and the adjacent alveolar walls are similarly thickened, and the alveoli which have become squeezed and distorted in the areas of interstitial pneumonia thus formed show reversion of the epithelial cells to the embryonal type. The alveoli beneath the pleura are emphysematous, and their epithelium cuboidal.

There is a moderate emphysema throughout the non-consolidated areas of the lungs, the alveoli being filled with swollen and fatty epithelial cells, with but few leukocytes, or the alveoli contain a granular material resembling the alcohol precipitate of albuminous fluid. In places the material derived from the broken-down epithelium stains deeply with eosin. The emphysema is considered to be secondary to the obliteration of the bronchi by the bacterial colonies and exudate.

A few small areas composed of cells which have coalesced or become ill-defined, with small round or fragmented, elongated nuclei, are seen in the sections. Such foci resemble the areas of focal necrosis seen in infectious diseases.

Bronchial lymph nodes.—There is a moderate degree of anthracosis. The congestion of the blood-vessels and the capillaries is intense, and there is considerable exudation into the trabeculæ and capsules of the nodes. The centres of reproduction are enlarged, and the proliferation, especially of the small, round, lymphoid cells, is marked. The lymph sinuses are compressed and show but little desquamation of their lining endothelium. No streptococci or other bacteria are demonstrable in the lymph nodes.

In general the lesions consist of an acute exudative and necrotic inflammation of the bronchi and surrounding alveoli; a broncho-pneumonia, associated with a productive inflammation of the framework of the lungs and organization of the alveolar exudate—an interalveolar and intraalveolar pneumonia. Apart from these lesions, there is an interstitial inflammation and thickening of the pleura and interlobular septa, which perhaps is more chronic in character.

CASE II. *Clinical History.*—F. F., aged 23, a laborer, entered Roosevelt Hospital, in the service of Dr. Delafield, January 13, 1899. His mother died of pneumonia. He was a moderate drinker. No syphilis or rheumatism. He has had measles, scarlet fever, diphtheria, and

Pott's disease of spine (?). He has had a chronic cough, since his tenth year, with muco-purulent expectoration and night sweats for several years (?). His present illness began about Thanksgiving day, 1898 (?), when he began to lose flesh and strength, and the pulmonary symptoms became more aggravated and were accompanied by dyspnœa. After Christmas, 1898, he became worse, complaining of headache, anorexia and general malaise followed by chills and a febrile movement, profuse sweating, increase of cough and expectoration, and pain in the chest.

On admission: Moderate emaciation and prostration. There was scoliosis of the dorsal vertebræ. Temp. 101° F.; Pulse, 112; Resp. 28. Examination of urine was negative.

Physical examination: Coarse breathing over left lung in front and behind, with mucous râles. Over lower part of right lung, behind, flatness, cavernous voice and breathing, gurgling and mucous râles. In front, breathing is coarse and high pitched, and there are many coarse râles.

In the hospital he became rapidly worse, with frequent cough and abundant expectoration, his breathing rapid and labored, and death followed pulmonary œdema on January 17, 1899.

Autopsy (Dr. E. Hodenpyl), 24 hours after death: The pleuræ of both lungs are covered in places with a fresh layer of fibrin. Both lungs are largely consolidated, the right lower and the left upper lobes being entirely consolidated. The left lower and the right upper lobes present the lesions of a broncho-pneumonia. The bronchi of both lungs show bronchiectatic dilatations, and numerous grayish actinomyces granules adhere loosely to the congested mucous membrane. On section the surface of the consolidated areas appears smooth and is studded throughout with grayish spots, which correspond to the cut sections of the bronchioles or smallest bronchi.

The bronchial lymph nodes are much enlarged and congested. On section, the nodes appear intensely hyperæmic and studded with small grayish foci.

No noteworthy lesions were observed outside of the respiratory organs.

HISTOLOGICAL EXAMINATION.—This, as in Case I, was limited to the lungs, which were preserved in alcohol. The lesions in the lungs are strikingly similar to those of Case I.

Bronchi.—Many of the smaller and larger bronchi are the seat of a catarrhal inflammation. The bronchial walls are congested and infiltrated by a serous exudate, with polymorphonuclear leukocytes, and the lumina contain leukocytes, fibrin and granular detritus. The adjacent

peribronchial alveoli are filled with leukocytes and desquamated epithelial cells.

The bronchial "granules" or colonies are composed of a felted network of filaments and rods, identical in appearance with those in Case I.

The bronchi are invariably necrotic where the granules are attached, or the necrosis implicates the whole of the bronchus and its adjacent alveoli. The necrotic inflammation is most marked in the walls of the dilatations, where all traces of the normal bronchial structure have disappeared. The cellular exudate in the bronchi adjacent to these colonies is necrotic, the leukocytes being transformed into a granular mass staining faintly with eosin. The largest bronchiectases are about a third of an inch in diameter, fusiform or sacculated, or irregular in outline. The alveoli around the dilatations are compressed and filled with fibrin almost to the exclusion of leukocytes. The fibrin is less compact and dense than in Case I.

Pulmonary parenchyma—The peribronchitic zones of pneumonia present a variety of appearances and consist of alveoli filled with leukocytes and a little fibrin. The alveolar walls are somewhat thickened by a cellular exudate, composed of cells of the epithelioid type, and the network of fibrin encloses a few epithelioid and polyhedral cells. The organization of the fibrin is in places well advanced.

As in Case I there are extensive areas of interalveolar or interstitial pneumonia. A few giant cells apparently formed by coalescence of the alveolar epithelial cells are found in the distorted alveoli enclosed by the new tissue.

The pleura is covered in places with a layer of fibrin and leukocytes, and is considerably thickened by a new growth of œdematous tissue. The blood-vessels are much congested. The exudate on the surface is more or less organized. Throughout the lymph spaces of the pleura and in the fibrin streptococci are found.

The interlobular septa and the alveolar walls adjacent to the pleura and septa are considerably thickened by a new growth of tissue composed of epithelioid and polyhedral cells and a homogeneous or slightly fibrillated basement substance, and the lining epithelium of the compressed alveoli has undergone reversion to the embryonal type.

The alveoli outside of the areas of broncho-pneumonia are somewhat emphysematous and contain large desquamated epithelial cells, which are markedly fatty or vacuolated.

From an examination of a large number of sections, the impression is gained that with the presence of the streptothrix colonies in the bronchi, the catarrhal inflammation is followed by a necrotic one.

No intra-alveolar hæmorrhage has occurred. The blood-vessels throughout the lungs are greatly congested, and the adventitial coats of the large vessels are markedly thickened and œdematous. No thrombosis was noticed, and the intravascular fibrin formation is moderate.

The *bronchial lymph nodes* show the same condition of acute inflammation as in Case I. Scarcely any pigment and no bacteria are found in the sections.

The lesions here, as in Case I, consist of an acute exudative and necrotic bronchitis, with bronchiectasis, a broncho-pneumonia, composed of alveoli filled with a cellular or fibrinous exudate, which is more or less organized, and a productive inflammation and thickening of the framework of the lungs—alveolar walls, interlobular septa and pleura.

The sections of the lungs of both cases are so similar that the cases can be distinguished from one another only by a comparison of several sections. The exudative phases of the inflammation in the bronchi and broncho-pneumonic areas and the proliferation of the alveolar epithelium in the non-consolidated areas are more marked in Case II than in Case I. The interalveolar and intra-alveolar pneumonia and the changes in the pleura and septa are more advanced in Case I. Case II, therefore, represents an earlier and less advanced stage of a lesion identical in its histological characters with that of Case I.

DESCRIPTION OF THE FILAMENTOUS STREPTOTHRIX COLONIES OR GRANULES AND OF THE BACTERIAL MORPHOLOGY OF THE LUNGS.

The gross appearance of the colonies in the bronchi as seen at the autopsies, the bacteriological examination of the coverslips and of the sections, and the cultural findings of the lungs of both cases may for convenience be considered together, as they were similar.

In the larger bronchi of both cases, either free or lightly attached to the congested mucous membrane, were found numerous opaque whitish and soft masses varying in size from 3 to 5 mm. In the bronchi of smaller caliber and throughout the necrotic and consolidated portions of the lungs smaller yellowish-white particles were scattered.

The colonies consist of a network of filaments, whose terminal portions are often bulbous. No distinct bulbs or end capsules were demonstrable in the smears or sections.

Examination of coverslips made from the colonies, stained by aqueous solution of fuchsin and by Gram, reveals an immense number of rods and filaments, with many cocci.

The cocci, nearly uniform in size, are found single, in pairs, or in short chains. They stain by Gram's method even after long decolorization in alcohol. Capsules could not be demonstrated by Welch's, Friedländer's or Gram's methods, nor by hot carbolie fuchsin.

The rods and filaments.—The various forms reveal a general similarity in morphology, but vary considerably in length, the shorter forms or rods being 6-10 μ in length, and the longer forms or filaments, two or three times as long as the rods. Many of the rods and filaments are more or less curved, have rough or irregular edges and are pointed or bevelled at one or both ends. Very slender and long filaments staining poorly were found, especially in Case I. The forms with rough edges frequently refuse to stain. The rods and filaments stain by Gram's method, but those which show irregularity in contour often decolorize. All the forms are broader than the tubercle bacillus.

The filaments stain faintly but fairly uniformly with aqueous solutions of methylene blue and of fuchsin. The rods stain irregularly and resemble closely those which were finally cultivated. No branching forms were positively recognized. In the coverslips made from other portions of the lungs the rods are much less numerous and do not predominate over the cocci. To contrast the bacterial morphology of the cases, the filaments on the coverslips of Case II stain more irregularly and are more beaded.

The impression gained after an examination of the coverslips of both cases, is that the rod and filamentous forms are merely variations of one microorganism. No tubercle bacilli were found.

THE BACTERIOLOGICAL EXAMINATION OF THE LUNGS OF BOTH CASES.

Sections.—The streptothrix colonies and cocci are best studied in the sections stained by Weigert's method of staining bacteria.

The filaments have no definite arrangement in the colonies. The streptococci are found in groups or short chains intimately mixed with the filaments. The necrotic cellular exudate in the bronchi contains numerous filaments, many of which are faintly stained and easily overlooked in contrast to the well stained.

A few streptococci and isolated filaments are found in the exudate of the catarrhal bronchitis and in the alveoli filled with fragmented leukocytes and organizing exudate. Where the exudate is mainly fibrinous

or composed of proliferated alveolar epithelium and granular material, no microorganisms are found.

The streptothrix filaments with a few streptococci are present in the necrotic bronchial walls and in the adjacent areas of new tissue, which shows nuclear fragmentation.

Streptococci alone are found in the lymph spaces of the bronchi, interlobular septa, and pleura.

With Sterling's gentian violet the filaments stain intensely and are beaded. In sections stained by this method and by carbolic fuchsin, a similar distribution of the streptothrix filaments is observed.²

In the sections of the lungs of both cases stained by Gram's or Weigert's method some of the bronchial colonies, or portions of these, were stained a mahogany or reddish-brown color (Plate XIII, A). The peculiar color reaction is confined to the filaments, the cocci invariably staining blue. The coverslips had been preserved for several weeks before the reaction was observed in the sections and the reddish-brown color fades from the sections in several weeks. The reaction is due to the iodine, as it is found in the coverslips and sections stained and examined in Lugol's solution. Although many of the rods on the coverslips did not give the iodine reaction, it was not confined to any one form of rods. The long, thin, and the irregularly edged filaments stain reddish-brown with noticeable frequency.

The substances known to take this color by iodine solutions are glycogen and the erythrodextrines. Some of the yeast fungi found in healthy stools are also said to stain red. A similar reaction has been noted in certain spore-forming butyric-acid bacilli.

Cultures from the lungs.—In both cases cultures were made at once from the colonies in the bronchi and from various portions of the lungs. Numerous pour- and streak-plates of glycerin and ascites-serum agar were made and grown under aërobic and anaërobic conditions. The ascites serum was slightly alkaline to test paper, the reaction of the glycerin agar was 1.5% acid to phenolphthalein. The original and the dilution plates, none of which were overcrowded, developed colonies which on inspection with a low power appeared identical, and coverslips revealed diplococci and streptococci staining by Gram. The rods were seen only when the solid particles carried over onto the original plates were employed as material for the coverslips. Numerous transplantations

² In sections stained by Sterling's gentian violet, the areas of interalveolar pneumonia contain ovoid cells resembling mast-cells and fibroblasts with brilliantly red protoplasmic granules smaller than the eosinophilic granulations of leukocytes.

from the particles (composed mainly of rods), on the original pour-plates, upon fresh media developed streptococcus colonies.

Thus on the ordinary culture media, glycerin and ascites-serum agar and also on Loeffler's blood serum, the streptothrix filaments failed to grow, while from both cases a similar streptococcus with the following cultural characteristics was alone isolated: Broth becomes turbid with a moderate whitish deposit resembling a pneumococcus broth culture more closely than a short-chained streptococcus. On potato no growth at 37° C. In gelatin stabs isolated colonies develop slowly along the puncture. Smears made from the broth culture reveal short-chained streptococci and diplococci. Subcutaneous inoculation of the broth streptococcus cultures and of the pus and colonies of the lungs of both cases in mice were negative.

INOCULATION OF ANIMALS WITH THE STREPTOTHRIX COLONIES AND PUS FROM CASES I AND II.

CASE I.—Three rabbits were injected intratracheally with a broth suspension of the colonies and pus. Two of the rabbits were killed two weeks later, with negative post-mortem results.

Rabbit, No. 3805.—This, the third rabbit, was injected with a small syringe-ful of the suspension on November 11, 1898, and died February 25, 1899, 63 days after inoculation. The animal is moderately emaciated. Autopsy reveals an extensive empyema of the left pleural cavity, the lung being compressed against the spinal column. The pus has a strong odor, and contains small whitish granules. Coverslips reveal cocci and numerous shorter and longer filaments, similar in morphology to those injected. Glycerin-agar pour-plates planted with the pus of the empyema, yield pure cultures of streptococcus. The mediastinal lymph nodes are enlarged, whitish and cheesy in appearance. Throughout the right lung, numerous whitish foci are scattered; the pleura appears normal. The other viscera are congested. The lungs were distended and preserved in alcohol.

Sections of the *right lung* show a general catarrhal and suppurative bronchitis and large peribronchitic zones of pneumonia. The exudate in the bronchi and alveoli is composed almost exclusively of leukocytes, and contains numerous streptothrix clumps. In the hæmatoxylin and eosin sections the clumps stain deeply with eosin, a few threads or filaments towards the centre being stained by hæmatoxylin. Stained by Weigert's or Gram's method, and by carbol fuchsin (Weigert), the peripheral filaments are seen to possess terminal swellings, and are radially

arranged, converging towards the centre of the clump. The rather dense zone of leukocytes attracted by chemotaxis around the clumps shows marked nuclear fragmentation. No streptococci or clusters of well-defined filaments are present in the lung. The clumps are smaller and do not resemble the streptothrix colonies seen in the bronchi of the human lungs, nor were such clumps seen in these lungs.

The pleura of the *left lung* is covered with a thick layer of necrotic exudate, containing small clusters, streptothrix filaments and streptococci. The pleura is much thickened by new tissue which extends into the adjacent and compressed lung tissue. A large cavity in the upper lobe is filled with a necrotic exudate. The wall of the cavity is composed of rapidly proliferating granulation tissue and of a fibrous zone of well-formed but œdematous connective tissue. The lung tissue around the cavity is compressed, and the alveolar epithelium shows marked reversion to the embryonal type.

There is a diffuse broncho-pneumonic consolidation, mainly of leukocytes, which exhibit marked nuclear fragmentation. There is a general suppurative bronchitis. Numerous streptothrix clumps are present in the exudate. Streptococci and groups of well-stained filaments are less frequently seen. The alveolar walls outside of the areas of consolidation are thickened by a cellular exudate, composed of polymorphonuclear leukocytes and many polyhedral cells or fibroblasts.

The intima of the larger and smaller blood-vessels is swollen, and there is considerable proliferation of its cells. The endothelium is well preserved. The muscular coats of the arteries have undergone hypertrophy, and the adventitia is thickened and œdematous.

The liver sections show a marked round-cell infiltration in Glisson's capsule. There is parenchymatous degeneration of the kidney and congestion of the spleen.

With the empyemal pus a second rabbit was injected intraperitoneally, February 25, 1899. Two weeks later it seemed ill and was emaciated; as death did not intervene, and the animal was recovering, it was killed April 6, forty days after inoculation. The autopsy showed a few whitish recent cicatrices on the peritoneum. With this rabbit, our last chance of isolating the streptothrix of the first case was lost, since as above stated, we had failed to secure a growth on artificial media.

CASE II.—Two guinea-pigs were injected in the peritoneum and two rabbits, one intravenously, the other through the trachea, on January 17. The bronchial material for all the injections was suspended in 1 cc. of broth.

Guinea-pig No. 3591 died January 24 with a general suppurative peritonitis. Many adhesions between the coils of intestine and retraction of the omentum are present. The mesenteric lymph nodes are enlarged, yellowish in color, the cross-section of the nodes resembling that of cheesy tuberculous nodes. Numerous small streptothrix granules are found free in the exudate or adhering to the serosa, and the examination of coverslips made from the pus reveals numerous cocci, and longer and shorter filaments and thin rods. Glycerin-agar pour-plates yield streptococci alone. The lymph nodes contain streptococci and filaments.

The *second guinea-pig* died January 24. In addition to a suppurative peritonitis, a serofibrinous pericarditis is found. The lungs and pleura appear normal. The viscera show intense congestion.

A rabbit injected intraperitoneally on January 24 with the pus of the first guinea-pig, was killed April 6. The autopsy was negative.

Rabbits.—A large rabbit, as above noted, was injected through the trachea on January 17, 1899, and killed January 30. Autopsy reveals an empyema of the right pleural cavity. The right lung is compressed and whitish in color and covered by fibrin. Examination of coverslips made from the pus show cocci, and filaments resembling in morphology those injected. The lungs are distended and hardened in alcohol. The pericardial layers are adherent. There is marked œdema of the mediastinal tissues, and the lymph nodes are enlarged, whitish and soft.

The left lung is studded with whitish foci. The other viscera are normal, except for coccideal areas in the liver. Sections of the left lung present a general catarrhal bronchitis, with small round-celled peribronchitic infiltration, mostly localized in areas resembling Arnold's lymph nodules. The whitish foci correspond to areas of broncho-pneumonia.

In the atelectatic or compressed portions of the *right lung*, the alveolar walls are thickened by a cellular exudate composed mainly of leukocytes, and by capillary congestion. There are extensive zones of peribronchitic consolidation, the alveoli and bronchi being filled with leukocytes. The leukocytes of the alveolar exudate have undergone nuclear fragmentation and the alveolar walls are in places infiltrated with leukocytes. Streptothrix clumps and a few isolated filaments with numerous streptococci are present in the exudate. The pleura is covered by a necrotic layer of fibrin and leukocytes, containing clusters of streptothrix and numerous streptococci. Beneath the thickened and necrotic pleura the lung tissue is compressed, and deeper a well-marked zone of new tissue has formed.

Sections of the heart and pericardium show a fibrinous and adhesive pericarditis; the fibrin has undergone hyaline metamorphosis and the pericardial layers and adjacent muscular fibres are necrotic, and infiltrated with leukocytes. Here and there in the myocardium beneath the necrotic layer, a well-marked round-celled infiltration exists. Streptococci are present in the exudate.

The blood-vessels throughout the right lung show more or less marked round-celled infiltration of their adventitial coats. The intima is regularly swollen, and in some arteries a well-marked obliterating endarteritis is made out.

Rabbit No. 3727, the second above mentioned, was injected January 17 in the ear vein with bronchial material of Case II and died February 14. Animal is much emaciated. The left pleural cavity is filled with whitish pus of the consistency of cream. The lung is compressed, the upper lobe being adherent to the ribs. The right pleural cavity is partly filled with a more fluid pus. The pleuræ of both lungs are covered with a layer of fibrin and pus. The other viscera of the rabbit are normal.

Coverslips made from the pus reveal cocci and filaments identical in morphology with those injected. Glycerin- and serum-agar planted with the pus yielded pure cultures of streptococcus. Numerous potatoes planted with the pus remain sterile. The organs of a rabbit were planted with the pus: on the kidneys streptothrix colonies developed, which are described in detail below under methods of isolation.

Histological examination.—The pleura is covered with a layer of necrotic fibrin and leukocytes with numerous streptothrix clusters. The superficial part of the thickened pleura is necrotic and infiltrated with leukocytes. The lung tissue adjacent to the pleura is compressed and infiltrated with small round-celled granulation tissue, extending in the lung to a variable depth from the surface and giving rise to areas of interstitial pneumonia. The alveoli enclosed in these areas assume bizarre shapes, and the alveolar epithelium may become columnar. Most of the sections of both lungs contain several larger and smaller abscesses and as no trace of bronchial structure remains even about the small abscesses, we are inclined to consider them metastatic abscesses.

Streptococci and clumps or clusters of ill-defined filaments are seen in the pleural exudate and in the abscesses where they are much less abundant. The walls of the cavities are formed by a layer of necrotic fibrin. Round-celled granulation tissue extends from this zone directly into the adjacent lung.

There is some dilatation of the bronchi, which in general show only

moderate degrees of catarrhal inflammation. Considerable intra-alveolar hæmorrhage has occurred about the abscesses. In some sections, the pneumonic exudate is extensive, and the infiltration of the alveolar walls has reached a moderate degree. The disintegrated tissue near the necrotic exudate abounds in nuclear fragments, and irregularly staining detritus, which in places have been taken up by large vacuolated or fatty cells.

A few circumscribed nodules composed of epithelioid cells, mostly disintegrated and with fragmented nuclei, occur here and there. Giant cells are not seen.

As regards the bacteriological examination of the sections, one is struck by the scarcity of the streptococci, and although the peculiar homogeneous clumps occur in moderate numbers, clusters of well-defined, or isolated filaments are but rarely seen. The clusters of filaments become transformed into the homogeneous eosinophilic clumps, which stain intensely with Weigert's fibrin method or by Gram. Transitional forms between the clusters of well-defined filaments and the homogeneous clumps occur. The most common one is a granular mass barely staining with hæmatoxylin or with anilin gentian violet, in which, however, a few filaments are definitely recognizable. Such transitional forms were found in all the experimental streptothrix lesions.

Besides the changes of the blood-vessels described in the previous rabbit, there is a general and well-marked hypertrophy of the muscular coats of the arteries throughout the lungs. The liver and kidney show acute congestion and slight parenchymatous degeneration.

The empyemal pus of the previous rabbit (3727) was injected into the ear vein of a rabbit (3752) and into the peritoneum of another rabbit (3806).

Rabbit 3752, intravenous injection of 1 cc. of the pus. Animal died 72 hours after injection.

Autopsy.—The peritoneum is intensely congested, and contains straw-colored fluid. The omentum is œdematous. The liver is congested and mottled. Kidney and spleen are congested. Through the pleura numerous small foci are seen. No cultures were made, as the autopsy was held 24 hours after death. The cause of death is considered to have been an infection with the streptococcus, perhaps increased in virulence by passage through the first rabbit.

Histological examination.—The lungs are highly congested. The blood-vessels in places contain the injected material. The emboli are composed of detritus, necrotic fibrin and leukocytes, with streptococci,

and a few homogeneous streptothrix clumps surrounded by a zone of leukocytes. Fibrinous thrombi, in places necrotic and without evidence of organization, occlude the blood-vessels. The coats of the blood-vessels are the seat of a necrotic inflammation extending to the adventitia, which is infiltrated with a serous exudate.

In the neighborhood of the thrombosed blood-vessels the alveoli are filled with fibrin, detritus and leukocytes, forming small areas of lobular pneumonia throughout the lungs. The numerous small white foci found at the autopsy, correspond to groups of several alveoli filled with leukocytes. In the larger areas the disintegration and infiltration with leukocytes of the alveolar walls are more advanced, the consolidation being composed of a mass of leukocytes, shrunken round cells and nuclear fragments, with necrotic fibrin. The liver contains numerous large areas of focal necrosis. The liver and kidney show parenchymatous degeneration, congestion and bacterial capillary emboli. There is an acute hyperplastic splenitis.

Rabbit 3806 was injected into the peritoneum with 1 cc. of the empyemal pus of rabbit 3727, on February 14 and died eleven days later. There is a general suppurative peritonitis, the coils of the intestines being matted together with fibrinous adhesions. The pus contains numerous cocci and filaments. The serosa of the liver, spleen and diaphragm is covered with a thick layer of fibrin. The mesenteric lymph nodes are enlarged, whitish and cheesy on cross section. The spleen is studded with small whitish foci.

Beneath the pleura of the lungs, numerous smaller and larger whitish areas are seen, the pleura itself appearing normal. The upper lobes of both lungs are completely, the lower lobes irregularly, consolidated. The upper left lobe contains a large cavity filled with whitish material and blood (Plate XII, Fig. 6). The bronchi of the right upper lobe are greatly dilated and contain pus, the consolidation of the lobe being whitish in color.

Histological examination.—The pathological process in the lungs presents the same phases of inflammation as in the lungs of the rabbits injected through the trachea, but the topography of the lesions is different, corresponding to their hæmatogenous origin.

The consolidated lobes contain wedge-shaped abscesses and areas of infarction, composed of alveoli more or less filled with leukocytes, fibrin, and a necrotic material, surrounded by a dense zone of leukocytes (Plate XII, Fig. 7). At the apex of the wedges the central blood-vessel is obliterated by a fibrinous thrombus which is necrotic and infil-

trated with leukocytes. The necrotic inflammation extends through the coats of vessels into the surrounding tissues, which are disintegrated and filled with nuclear fragments. A layer of necrotic fibrin surrounds the zone of pus cells, and the adjacent lung tissue is infiltrated with small round cells, an early stage of limitation or demarcation by fibrous tissue.

Numerous smaller abscesses without apparent connection with the blood-vessels are scattered throughout the lung. The upper lobes, are diffusely consolidated, the bronchi are markedly dilated, and the seat of a mild catarrhal bronchitis, with desquamation of the epithelium. In the lower lobes, there is broncho-pneumonia with suppurative bronchitis. The tissue surrounding the largest bronchiectases, is extensively infiltrated with blood.

The coats of the blood-vessels are the seat of an exudative inflammation most marked in the adventitia, and fibrinous thrombi obliterate some of the vessels. There is a general round-celled periarteritis of the smaller vessels. New tissue is found around the bronchial dilatations in the form of areas of interstitial pneumonia. The lungs in other situations are atelectatic and the alveoli contain desquamated cells and granular material.

The serosæ of the liver and spleen are thickened and covered with a layer of fibrin and pus cells. Numerous streptococci, a few clumps and clusters of streptothrix, and isolated filaments which give the reddish iodine reaction are found in the layer of exudate; the liver and kidneys show acute congestion and a moderate degree of parenchymatous degeneration.

The Malpighian bodies of the spleen are enlarged, the blood-vessels are congested, and contain many leukocytes. The endothelial cells of the capillaries show proliferation and degeneration, and a fibrinous exudate is found beneath the capsule.

To summarize briefly, the introduction into the lungs of rabbits through the trachea of the bronchial material of Case I and Case II, consisting of streptococci, streptothrix rods, pus cells, etc., was followed by suppurative and necrotic broncho-pneumonia with bronchiectases, pulmonary abscesses, and extension of the inflammation to the pleura and pericardium with empyema.

Inoculation of the infectious bronchial material of Case II into the ear vein of a rabbit gave rise to pulmonary abscesses, diffuse pneumonic consolidation of the lungs and empyema. The empyemal pus

of this rabbit injected into the peritoneum of another rabbit produced a suppurative peritonitis with metastatic pulmonary abscesses.

In guinea-pigs, inoculation of the bronchial material of Case II into the peritoneum produced suppurative peritonitis.

In the rabbit the pathological process was acute, the leukocytic infiltration and disintegration of tissue led to the formation of abscesses with well-marked limitation of the process by a zone of demarcating connective tissue.

THE METHODS OF ISOLATION, AND THE MORPHOLOGICAL AND BIOLOGICAL
CHARACTERS OF THE STREPTOTHRIX OF CASE II.

Methods of isolation.—The usual laboratory media having been found unsuitable for the cultivation of the streptothrix, the method of streaking the fresh organs of rabbits was resorted to. The organs of normal rabbits, immediately after killing, were transferred to Petri dishes, precautions being taken to avoid contamination during removal. The kidneys were halved. The various organs were planted with the empyemal pus of rabbit 3727 and the Petri dishes placed in covered chambers containing sterilized water to prevent drying of the organs. After two days at 37° C., six elevated whitish colonies, the largest 2-3 mm., developed on the kidneys. On the other organs—liver, spleen, heart and muscle—no growth was visible, but streptococci were found on the coverslips, and recovered in pure culture from these organs. Coverslips prepared from the colonies on the kidney showed rods resembling in morphology the bacillus of diphtheria, mixed with moderate numbers of cocci. The average length of the rods approximates that of the tubercle bacillus; they are somewhat broader than this bacillus. The ends are frequently bulbous, less often distinctly wedge-shaped. Some of the rods are frayed out at the end, an appearance suggestive of fragmentation and not common to bacilli. No indication of branching is found, although it was searched for. Stained with methylene blue, the rods appear beaded or striated. The staining reactions and morphology of the growth on kidneys will be more fully described below.

The kidney growth was planted in broth and on the various organs

of a fresh rabbit. The broth tubes yielded pure cultures of the streptococcus. Again on the planted halves of the kidneys, but on none of the other organs, a growth of rods was obtained. Successive transplantations upon fresh kidneys, and also on the other organs, of rabbits were then made at intervals of three days. In no case did the rods grow, as determined by smears, on any organ but the kidney. A photograph (Plate XI, Fig. 5) shows colonies of the streptothrix growing on the rabbit's kidney.

The second generation on kidney was an abundant one, numerous colonies developing along the course of the streaks. Coverslips of the growth revealed the rods, morphologically speaking, in pure culture. Broth, however, planted with the growth again yielded a pure streptococcus culture. The third generation on kidney was still more luxuriant, the growth forming confluent and raised masses, glistening slightly, and resembling in texture the substance of yeast cakes. The later generations on kidneys were moister and diffusely scattered over the surface. The morphology of the third and later generations differed somewhat from that of the first growth, in that the rods were longer and more frayed out at the ends. The wedge-shaped rods were observed only in the early kidney growths.

The striking predilection of the rods for the kidney as a medium of cultivation, and the complete absence of growth on the other organs of the rabbit, is a fact of singular importance. Many observers have recorded observations on the special suitability of various organs for the growth of a variety of microorganisms, but so far as our knowledge extends, no such striking predilection for a particular organ has been noted.

After several generations on kidney had developed we were somewhat perplexed how to proceed further in our study of the rods which were found later to be a species of streptothrix. The streptococcus was still present as revealed by broth cultures in the third successive kidney growth, and the rods still refused to grow in broth, at least in symbiosis with the streptococcus. The growth of the streptothrix on kidney being assured, methods for separating the two microorganisms engaged our attention. Three methods were tried: First, by taking advantage of a

possible difference in the thermal death point of the two microorganisms. The growth on the kidney was suspended in a number of broth tubes which were subjected, some to a temperature of 50° C., others to 56° C. for ten minutes. A number of trials at both temperatures were made. Both microorganisms failed to survive the exposure, transplants failing to produce a growth on broth and on kidney. The second method, prolonged cultivation from kidney to kidney, the transplants being made after three days' growth at 37° C., we were led to believe would succeed, from a comparison of the coverslips taken from the early generations on kidney after several days' growth at 37° C. and later when the kidney growth was kept at room temperature. It was observed that the rods overgrew the streptococcus at incubator temperature, the coccus first becoming visible on the smears when the cultures were kept at room temperature, when the rods, as was determined later, cease to grow below $28-30^{\circ}$ C. approximately. The sixth generation on kidney controlled by broth cultures was a pure streptothrix culture. The continuous kidney cultivation was forced upon us, as at first the rods failed to grow in our broth, a fact which did not encourage us to try the next and third method of separation by washing the mixed kidney colonies, which, however, led to success. A well isolated colony was looped, and shaken in successive broth tubes. Five broth tubes and kidneys were finally planted with the washed bits of growth. The kidneys and one of the broth tubes yielded pure cultures of the streptothrix; one tube gave a mixed culture; the others, a pure streptococcus culture. The broth used for all came from the same stock of beef peptone broth, 1.5% acid to phenolphthalein.

The first broth growth was meagre, and with it glycerin and serum agar plates and broth tubes were planted. Again the broth alone showed a growth. Anaërobic stabs in four raw eggs were tried. In one egg several masses composed of a felted clump of mycelium developed but the cultivation in eggs was considered uncertain and was abandoned.

The persistent refusal to grow on any organ but kidney, or on solid media, had suggested the advisability of trying broth and agar in which kidney infusion replaced the usual meat infusion. Broth and 1.5% agar were made both with human and lamb kidneys, with the usual addition of peptone and salt. The reaction was found neutral to test paper or about 0.4 acid to phenolphthalein. In plants of the media with the mixed growth on kidney the streptococcus alone developed.

It was thought possible that, as in the case of the gonococcus, uncoagulated proteids were essential for growth. Chopped rabbits' kidneys were

added to fluid agar at 43° C. planted and poured. The streptothrix failed to grow, however. The following media were also planted, all with negative results: Agar and broth plus sterilized urine; aseptic serum agar, slightly alkaline, with and without glycerin; Loeffler's and coagulated calf's blood serum; prune juice media with peptone and salt of different reactions, alkaline, neutral, and slightly acid; semi-solid mixtures of gelatin and agar, and Hiss's typhoid plating medium.³

Broth cultivation of the streptothrix was pursued for two months, transplants being made at intervals of a week, the growth becoming more vigorous and abundant in each successive culture.

Plating media.—From the eighth generation on broth a growth was finally obtained on solid media—the usual peptone agar with addition of 5% glycerin, made to react neutral or 0.5% acid to phenolphthalein.

Separate portions of the agar media with and without addition of 2% glucose were made to react 0.5 alkaline, neutral, and 0.5 to 1% acid to phenolphthalein. As the acidity is decreased, the plating media become softer. To insure accuracy, each set of media was tested after the final sterilization, and two original and one streak plates were planted with large loopfuls of the eighth generation on broth.

In the first and second series of plates those of 0.5 acid reaction furnished the most abundant growth, the other plates containing only several colonies. In a third series the most abundant growth was obtained on the plates of neutral reaction; several colonies now grew on the media of 1.5% reaction.

At the maximum only 50 to 60 colonies developed on the original plates, in marked contrast to the overcrowded pour-plates planted with bacterial cultures.⁴

Description of the streptothrix colonies on neutral or 0.5 acid glycerin agar.—The colonies are visible at the end of 48 hours at 37° C., and attain their maximum development in the course of a few days. The largest are 3 to 4 mm. in diameter. The growth resembles more nearly that of bacteria than the dry growth described as characteristic for many

³ Hiss, *Journal of Experimental Medicine*, 1897, ii, p. 677.

⁴ The following explanation of the scanty growth on solid media is suggested: Though spores were never observed, the sporulation of the streptothrix in the broth culture may be reduced to a minimum, and thus have escaped observation. The colonies may arise only from the germination of the few spores and the subsequent rapid increase in length of their mycelia; whereas the non-spore-bearing streptothrix rods, which are in the majority after the fragmentation caused by the transfer from broth to solid media, stop growing, or grow so slowly that no colonies visible to the naked eye or under a low power develop. However this may be, solid media, barring the kidney, are not suitable or favorable soil for this streptothrix.

of the *Streptothrices* and allied microorganisms, the tubercle bacillus, etc.

The deep colonies are pin-head or smaller in size, whitish in color and opaque. Spreading colonies are formed out of these deep colonies by extension of the growth into the medium, or on the bottom of the plate. The borders of these so-called disseminated colonies are made up of smaller colonies or groups of rods which lend them a characteristic appearance. The central opaque portions of the colony resemble the non-spreading deep colonies.

Stab cultures on agar (1.5% acid reaction) planted from the colonies now yielded a fairly abundant growth of discoid and isolated colonies along the puncture, the largest ones being 2 to 3 mm. in diameter, whitish in color and sharply defined.

A scanty, whitish growth was now obtained on coagulated ascitic serum containing broth and sugar, and on Loeffler's blood serum. The colonies adhere firmly to these media in contrast to the loose growth on the kidney and on moist glycerin agar. Firm adherence to the surface of media is characteristic for many species of streptothrix, being due to the penetration of the growth into the depth of the media.

The growth in *broth* resembles the granular variety of a streptococcus culture, the small discoid granules adhering firmly to the sides of the tubes, leaving the broth clear. The differences observed between the early and the later broth cultures are due to the more abundant and rapid growth of the streptothrix as cultivation proceeded. In the later cultures a sticky sediment was constantly present after a few days of growth, the granules becoming larger and fluffy with the increase in size and then sinking to the bottom after slight handling of the tubes.

Two varieties of broth culture occur, the one described above with small, hard and adherent discoid granules, or larger fluffy particles; the other the large clumpy variety of broth culture. The clumpy variety is characterized by the formation of a few large clumps at the bottom of the tube which slowly increase in size; the clumps are dull gray in color, firm and gritty. It was observed in a few of the early broth cultures and later in some broth tubes, which were planted with the cheesy material of the lymph nodes, etc., of rabbits that had been injected subcutaneously with pure cultures of the streptothrix. The clumps are seen to form directly from the bits of cheesy material, which slowly increase in size after 8 to 12 days in the incubator at 37° C.

Fermentation tubes.—In litmus 2% grape-sugar broth, no gas forms. The growth develops equally well in both arms of the tube. The color

begins to change 24 to 48 hours after the growth first becomes visible, the production of acid being slow. Discoloration soon occurs and is most marked in the closed arm. Grape sugar does not increase the rapidity of growth in broth. Other sugars were not tried.

Milk cultures.—In litmus milk after 48 hours, the color changes, soon becoming markedly red. Clotting usually occurs on the fourth day or may be delayed to the fifth or seventh day. The clot is soft, finely divided and occupies the whole of the fluid. The purity of the milk cultures was tested by transplants into broth.

In order to determine the cause of the clotting broth cultures were filtered through sterilized filters and added to sterilized milk. No coagulation of the milk occurred after addition of a few drops up to several cc. of the filtered broth culture, either at once or after several days at 37° C. The coagulum is apparently caused by acid production, no soluble enzymes being demonstrable.

Several determinations of the acidity of broth and milk cultures, after 7 to 10 days' growth, were made. Broth was found to have increased in acidity from 1.5% acid to 4.5% and 6% acid to phenolphthalein.

In Dunham's peptone solution of various degrees of reaction no growth occurs, and on plain or glycerinated potatoes, tested and found to be only a few tenths of one per cent acid, no growth occurs, as confirmed by smears.

The absence of growth on potato is not in consonance with the well-recognized suitability of the potato for the cultivation of many *Streptothrices*. Potato culture is said to favor the formation of the aërial and spore-bearing hyphæ of a number of species of this genus (*Streptothrix Eppingeri*), and a characteristic musty or mouldy odor accompanies the sporulating stage of growth. *Streptothrix Israeli* (?) and a few other species fail likewise to grow on potato.

Vitality.—The streptothrix is short lived. The longest period it was found capable of retransplantation from broth to broth was three weeks. Grown on kidney at 37° C. for four days, and then kept for two weeks in a moist chamber at room temperature, when further growth ceases, successful transplantation on fresh kidney becomes uncertain.

Optimum temperature.—It grows best at 37° C. In the summer months when the temperature ranged at times from 24° C. to 29° C., a few tubes of broth and gelatin showed a slight growth on the fifth or sixth day.

In broth, the streptothrix grows equally well in the absence or presence of oxygen, and no change in morphology was observed in the anaërobic growth. The growth on all media is odorless.

Morphology of the streptothrix.—The morphology of the streptothrix is well shown in the photomicrographs (Plate XI). The streptothrix on the coverslips made from the bronchial colonies and pus, and from the lesions and pus of rabbits and guinea-pigs induced by these, presents rod-like or filamentous forms; no branching forms or bulbs, after appropriate staining or in the fresh condition, were identified.

In the growths on the rabbit's kidney, it still retains its rod-like form, but is somewhat shorter, and closely resembles bacilli belonging to the pseudo-diphtheria group. The club-shaped or wedged ends and bulbous central portions of the rods stain intensely with the dyes, and with aqueous solution of methylene-blue marked metachromatism is present. The grouping in closely approximated and parallel lines is frequent. In the later generations on kidney the ends of the rods become poorly defined or taper off gradually, and stain faintly, the first indications of fragmentation (Plate XI, Fig. 1).

The irregularity in staining with methylene blue and aqueous fuchsin solutions gives rise to a beaded appearance. When dilute solutions of dyes slowly penetrate under the cover glass, the bulbous ends and the round refractive swellings stain more intensely than the rest of the protoplasm; in some places the rods remain unstained, or only a portion of the refractive swellings may stain. The bulbous ends stain deeply with Delafield's hæmatoxylin, whereas the rest of the protoplasma is scarcely tinged.

With P. Ernst's stain (methylene blue and Lugol's solution), every rod possesses one or several intensely black, round spots; these are usually single, situated at one extremity or at both ends, or irregularly distributed in the protoplasm, which stains a faint yellow (Plate XI, Fig. 4). These bodies are often smaller in diameter than the breadth of the rod.

No branching forms were discovered in the growth on kidney, either in the coverslip preparations or in the sections of the growth on kidney. Branching was first positively recognized in the early generations in broth (Plate XI, Fig. 4), the fragmentation of the filaments and the branching increasing with the further cultivation in broth.

As shown in the photographs there are striking differences in morphology obtained after different staining methods. Figures 2 and 3 (Plate XI) are of smears made from the same colony on glycerin agar, stained respectively by methylene blue and by Gram. Figure 2 shows the beading of the filaments and large numbers of small rods or coccus-like forms. In Figure 3, from specimens stained by Gram, these features are not prominent, the staining being diffuse, intense, and only a few

short rods are seen. The contrast in the morphology⁵ after the two stains is so striking that we believe many have fallen into error when describing the morphology of various streptothrices and have laid too much stress on such coccus-like forms, which have been often mistaken for spores. By careful focussing the connecting links between the colored portions of the rods (stained by methylene blue) can be recognized. Stress is laid on these observations on account of the frequent neglect of most observers to mention the stains employed.

The viscid deposit in the broth tubes seems to be largely intracellular substance, mixed with rods which stain faintly. Curious forms resembling stalks were observed when this material was examined. They varied greatly in size, and their branches form an intricate network, the meshes of which vary in size from delicate filaments up to very broad stalks.

When stained by methylene blue or by P. Ernst's method, the relative position of the darkly-staining spots in the stalks to each other would seem to indicate that stalk formation occurs by the coalescence of the filaments along their length. Sauvageau and Radais, who suggest this explanation, give an excellent description of similar stalk formations in the two species of streptothrix they described.

Our streptothrix in broth and milk cultures exhibits extreme pleomorphism. There are short rods with bulbous extremities, longer rods with marked beading, or with considerable unstained protoplasm. In milk the short rods, and those with bipolar staining are especially common; and long, thin and curved filaments, with or without irregular beading, resemble the filaments of the bronchial colonies.

The short rods with bipolar staining from the milk cultures closely resemble those recovered from the cheesy lesions of rabbits after subcutaneous injection with pure cultures. The scarcity of such forms in the lesions, and the difficulty of recovering the streptothrix in cultures, unless large loopfuls of the cheesy material are transferred, incline us to regard these as degenerative forms.

Morphology of the streptothrix in the experimental lesions.—In the lesions of the guinea-pigs and rabbits produced by the inoculation of the bronchial material of Cases I and II, the streptothrix was present in large numbers in the pus of the pleura and peritoneum together with numerous streptococci. In morphology the rods and filaments resembled closely those composing the streptothrix colonies of Cases I and II.

⁵ Bostroem with the *Actinomyces bovis* and Aoyama with the species of streptothrix which he has lately isolated, have called attention to this contrast in size presented by streptothrix after various staining methods.

In the pulmonary lesions produced by intratracheal introduction of pure cultures into rabbits the streptothrix is found in clumps, which undoubtedly arise from the discoid granules of the broth cultures, and only a long search reveals a few isolated filaments. In the lesions of the skin or lymph nodes after subcutaneous injections a few clusters of streptothrix filaments or rods with bipolar staining may be found.

The clumps stained by hæmatoxylin and eosin are strongly eosinophilic and homogeneous. The fine radiations which extend from the periphery to the centre of the clump when stained by Mallory's⁶ method or by carbolic fuchsin (Weigert) appear as long and variously-shaped bulbs or terminal swellings, which extend into the zone of leukocytes surrounding the clump. In every clump a few distinct bulbs enclosing central well-defined mycelial filaments are found.

In contrast to their faint staining by these methods, the bulbs and terminal swellings stain intensely by Weigert's fibrin-stain (Plate XIV). As a few clumps were found in the bronchi and fibrinous pleural exudates of the rabbits injected with the bronchial material of Cases I and II the streptothrix filaments under certain conditions evidently become transformed or agglutinated into clumps, similar to those derived from the granules of the broth cultures. The few finely granular clusters of more or less disintegrated and faintly-staining rods seem to be transitional forms which may later develop into clumps.

The intense staining of the terminal swellings of the mycelial filaments in the clumps when stained by Weigert is well shown in our drawing (Plate XIV). They closely resemble the bulbous forms of the tubercle bacillus as depicted by Babes and Levaditi in their article on the actinomyotic form of the tubercle bacillus and also by Lubarsch and by Schultz in their articles on the bulbous forms of the tubercle bacillus and other bacteria. For a discussion of the nature of the bulbs, etc., which cannot be entered upon here, we refer to Lubarsch.

The morphology of the streptothrix remains the same when grown in collodium sacs within the peritoneal cavity of the rabbit.

The original smears of the bronchial colonies had been kept for several weeks before being stained by P. Ernst's method and then no chromatic particles were seen. To determine whether this was due to prolonged drying of the smears, even after the usual fixing, smears from broth cultures were kept after fixing by heat for several weeks. They were then stained with hæmatoxylin, by Ernst's method, and by

⁶ Mallory and Wright, *Pathological Technique*, p. 282. Method No. 1. Philadelphia, 1897.

methylen blue. The rods invariably stained faintly, and no granules were found. Prolonged drying changes apparently the staining properties of the plasma. Such observations, however, do not aid in determining whether the granules are nuclear or merely reserve depots of material, but perhaps explain their absence from the original smears.

Curious forms characterized by marked tenacity for retaining the stain after decolorization in alcohol were observed in the later generations on broth. When the smears were stained by hot carbolic fuchsin and decolorized in 97% alcohol, followed by washing in water and drying, the intensely staining forms are bulbous, less often bacillary or coccus-like, or branched (Plate XIII, B). With a methylene-blue afterstain the rods stained blue and these questionable forms retain their brilliant red color. When weak solutions of acids are used, the decolorization is prompt and complete.

The morphology of the streptothrix growing on various solid media, Loeffler's and coagulated blood serum, etc., does not differ from that described above.

Growth in the hanging drop.—The streptothrix is non-motile. The branching of the delicate mycelial threads is seen to best advantage in the hanging drop. The irregular swellings and club-shaped ends of the rods, and the round refractive bodies, some apparently free, are also well observed.

The difficulty of watching the growth was increased, as the streptothrix does not grow at room temperature. In broth, aërogenous spore formation was not observed nor was actual fragmentation seen to occur. We were able to watch the actual increase in length of the small buds or projections from the rods and filaments into branches of considerable length. The observations entitle us to consider the branching a real one, and to separate definitely our streptothrix from the group of so-called pleomorphic bacteria. The trend of recent studies limits the usage of this epithet, to describe not a group, but merely a condition of pleomorphism in microorganisms.

The vast number of branching forms seen in smears made from pure cultures also justify us in considering our rods as a species of the genus *Streptothrix*.

EXPERIMENTAL LESIONS PRODUCED BY INOCULATIONS OF PURE CULTURES OF THE STREPTOTHRIX.

SERIES I.—The eight rabbits of this series were injected on March 27, 1899, either with the pure growth of streptothrix of the 6th generation

on kidney, suspended in broth, or with broth cultures obtained by washing the 3d generation on kidney, as indicated below.

Intraperitoneal injections: Small rabbit No. 1.—Kidney culture. The animal, moderately emaciated, was killed 42 days after inoculation. Whitish patches of thickening of the parietal peritoneum are found. In places the patches are depressed. Autopsy otherwise negative.

Small rabbit No. 2.—Broth culture. Animal emaciated, was killed 42 days later. Whitish patches somewhat more depressed but otherwise similar to those of rabbit No. 1, are found. Microscopic examination shows the patches to be formed of new fibrous tissue. Pea-sized yellowish nodules are found attached to the intestine and mesentery, and cover-slips prepared with the dry material reveal a few rods with a faint bipolar stain, but broth tubes planted with a loopful remain sterile. The cæcum contains coccidial ulcers which extend down to the peritoneal coat.

We believe the lesions described to be late or nearly healed stages of lesions produced by the streptothrix. Both animals received small quantities of culture.

Rabbit No. 3.—To compare the lesions following upon combined injections of the streptothrix and the streptococcus, with those produced by the streptothrix alone, a rabbit was injected in the peritoneum with 1 cc. of a (7th day) broth culture of the streptococcus isolated from Case II, together with the broth streptothrix culture. Animal killed 44 days later; no lesions were found.

Intravenous injections: Rabbit No. 4.—Kidney culture of the streptothrix. Animal killed 43 days after inoculation. Autopsy negative.

Rabbit No. 5.—Broth culture. Killed 43 days after inoculation, autopsy likewise negative.

Intratracheal injections: Rabbit No. 6.—Kidney culture. Killed 44 days after inoculation. Autopsy negative.

Rabbit No. 7.—Kidney culture. Killed 49 days after inoculation. Autopsy negative.

Rabbit No. 8.—Broth culture of streptothrix and streptococcus. The possibility that the streptothrix alone introduced into the lungs through the trachea might produce no lesions, led us to inject 1 cc. of a (15th day) broth culture of the streptococcus isolated from Case II, together with a broth streptothrix culture, into the trachea of rabbit No. 8. We conjectured that the streptococcus would predispose the lung to infection with the streptothrix. This rabbit, killed 48 days later, was the only one of the four animals injected into the trachea that presented lesions.

Sections of the lungs reveal many pin-head to small pea-sized masses. The microscopical examination shows these masses to consist of intrabronchial new-formed tissue. The centres of many of these masses contain a homogeneous body, staining deeply with eosin, made up of the discoid granules of the streptothrix broth culture, which we shall refer to as the streptothrix clump. The peculiar morphological features of these clumps, which were present in the lesions of all the animals of the next series, are described under Morphology (p. 179).

The clumps have attracted around them a more or less dense zone of leukocytes, which is surrounded by a layer of necrotic tissue composed of ill-defined cells (staining deeply with eosin) with long and bizarre shaped nuclei or merely nuclear fragments. The fragmented nuclei of these tissue cells are arranged in lines of chemotactic attraction radiating towards the centre of the clump. The peripheral portions of the masses are formed by a spheroidal or polyhedral-celled tissue. When the intrabronchial mass of new tissue is of large size the normal bronchial structure is no longer seen, and the periphery of the mass is encapsulated by tissue of a more fibrous type. The peribronchial or alveolar walls adjacent to the masses are more or less extensively infiltrated by spheroidal cells.

In places areas of new tissue resembling granulation tissue occlude the bronchi and these may be so large that their original connection with bronchi cannot be definitely made out.

Besides the intrabronchial masses enclosing the streptothrix clumps there is a general catarrhal bronchitis and thickening of the bronchial coats by new tissue composed of spheroidal and polyhedral cells with here and there nodular projections of the submucosa into the lumen of the tube covered over by the bronchial epithelium. The lymphoid nodules of the bronchi are hyperplastic.

The first series of inoculations were in the main negative, the sole exception being rabbit No. 8, which alone of the four animals injected into the lungs, received, in addition to the streptothrix, a streptococcus culture.

The lesions in this rabbit consisted of catarrhal bronchitis with diffuse and nodular newly-formed connective tissue—productive bronchitis—hyperplastic lymphoid nodules, and the intrabronchial masses of tissue formed about the streptothrix clumps, or discoid granules of the broth culture. As will be seen from the description of the lesions of the rabbits of Series II, similar and even more marked lesions follow introduction into the lungs of larger quantities of streptothrix granules ob-

tained from broth cultures. The negative results of this series may therefore be attributed to the small quantities of streptothrix granules injected.

SERIES II.—In this series of inoculations larger quantities of streptothrix granules were injected. The granules were broken up as finely as possible so as to minimize their action as foreign bodies.

With the object of predisposing the lungs to infection with the streptothrix, after completion of the operation for tracheotomy, in all but one of the rabbits, a solution of 2 drops of concentrated ammonia in 15 drops of water was injected into the lungs through the trachea, followed 24 hours later by the intratracheal injection of the streptothrix culture. After each injection the small wound was stitched, and in none of the cases did infection of the wound follow. During the intratracheal injections the animals were inclined to the right side.

Rabbit No. 10.—April 3, intratracheal injection of ammonia followed 24 hours later by one-half of the streptothrix granules of a broth culture. Animal killed 37 days later. Pin-head sized foci and larger areas 5 to 6 mm. in diameter, surrounded by an cedematous translucent zone of tissue, are scattered throughout the lobes of both lungs. The lower left lobe is reddish in color and consolidated. Microscopical examination shows catarrhal bronchitis with considerable exudate in the larger tubes. The bronchial, peribronchial and alveolar walls are infiltrated with spheroidal and polyhedral cells. In the mucosa, nodular intrabronchial projections of new-formed tissue are found, which in places have caused a partial or complete obliteration of the lumen of the tube. The tissue resembles cedematous and vascular granulation tissue, and a drawing shows the canalization of the tissue with spaces lined by bronchial epithelium (Plate XV). In this rabbit there are areas of pulmonary atelectasis, and of lobular or broncho-pneumonia, the exudate consisting of leukocytes, epithelial cells, nuclear fragments and a few small giant cells. There is some necrosis of the cellular exudate. The alveolar walls are infiltrated by a few spheroidal or polyhedral cells. The adventitial coats and the intima of the larger blood-vessels are cedematous. Besides these lesions, the smaller bronchi are occluded by intrabronchial masses containing the streptothrix clumps, similar to those described in rabbit No. 8.

The pulmonary lesions in this rabbit, therefore, consist of intrabronchial masses of new tissue, enclosing the streptothrix clumps, of broncho-pneumonia—obliteration of some of the bronchial tubes and areas of lobular pneumonia with atelectasis.

Rabbit No. 11.—April 20, intratracheal injection of the streptothrix granules of a broth culture (15 days' growth), without the preliminary injection of ammonia. Animal killed 20 days after inoculation. About the trachea are found several large yellowish masses surrounded by fibrous tissue, due to escape of the culture through the track of the needle.

Scattered throughout the lungs small pin-head foci (Plate XII, Fig. 8) most numerous posteriorly, are found, similar in their microscopic appearances to the intrabronchial masses around the streptothrix clumps described above in rabbits Nos. 8 and 10. No other lesions are present in the lung.

Rabbit No. 12.—April 4, intratracheal injection of ammonia followed 24 hours later by an intratracheal injection of half of the streptothrix granules of a broth culture (15 days' growth). Slight embarrassment to respiration followed both injections; animal killed 36 days later.

Small (5 to 6 mm.) flat masses surrounded by a translucent zone project beneath the pleura. The posterior portion of the lung, reddish in color, is consolidated by areas of lobular pneumonia. The intrabronchial masses around the streptothrix clumps are larger than those of the preceding rabbits. The largest masses situated beneath the pleura, which is congested, œdematous and thickened, are composed of a large central collection of pus cells and necrotic tissue, with an occasional streptothrix clump and are encapsulated by an extensive zone of well-formed spheroidal or polyhedral-celled tissue, which extends diffusely beneath the pleura into the adjacent lung tissue. A few arteries present an obliterating endarteritis. The lesions, judging from the larger size of the abscesses, are more severe than those of rabbit No. 10. The encapsulation by fibrous tissue testifies to the successful conservative attempt on the part of the lung to limit the process.

Rabbit No. 13.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules obtained from various broth cultures. Twelve days later the animal was killed.

At autopsy there are several large yellowish masses resembling inspissated pus, surrounded by a fibrous capsule in the peritracheal tissue due to escape of the injected fluid. There is a sero-fibrinous mediastinitis. The upper lobe of the right lung contains reddish, collapsed areas, and yellowish-white pea-sized masses project beneath the pleura. White pin-head sized masses project beneath the pleura. White pin-head sized foci are scattered throughout the lobes of the lungs.

The streptothrix was recovered in pure culture from the peritracheal and from one of the pleural masses in the upper lobe.

Microscopically the subpleural masses resemble those of rabbit No. 12. The foci consist of alveoli filled with spheroidal and epithelial cells. The right upper lobe is extensively atelectatic, and the alveoli contain exfoliated epithelium and large multinucleated giant cells. There are also areas of interstitial tissue of the ordinary type.

Rabbit No. 14.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules, obtained from the same broth cultures with which rabbit No. 13 was injected. Animal, much emaciated and with marked dyspnoea, was killed 12 days after inoculation.

The autopsy showed catarrhal bronchitis with much exudate. A large cavity with dense fibrous walls and filled with creamy semi-solid material occupies nearly the whole upper lobe of right lung. Numerous smaller cavities and white foci are scattered throughout the various lobes of both lungs. The pericardium and left lung are adherent to the chest wall and there is sero-purulent pericarditis.

Microscopically the cavities and abscesses are seen to be surrounded by extensive zones of oedematous granulation tissue. The lung tissue between the abscesses is atelectatic or consists of new-formed fibrous tissue. The pus in the cavity and abscesses contains numerous rods and cocci and a few streptothrix clumps. The necrotic walls of the cavity likewise contain cocci and rods. Cultures yield a variety of microorganisms which were not identified.

The lungs resemble those of the rabbits which were injected through the trachea with the streptothrix masses and pus obtained directly from the original human cases. The result in this experiment indicates that cavity formation and severer lesions are produced when there is concurrent infection with cocci. In this case the cocci were either introduced accidentally at the time of injection, or entered later from the air passages.

Rabbit No. 15.—May 12, intratracheal injection of ammonia followed 24 hours later by injection of the streptothrix granules obtained from various broth cultures. Animal killed 10 days later. Near the trachea two yellowish almond-sized lumps encapsulated by radially striated dense fibrous tissue are found. The lumps are due to the escape of the fluid at time of injection through the perforation in the wall of the trachea.

Upper lobe of right lung is dark red in color, consolidated, somewhat collapsed, and does not distend when the lungs are injected. Sev-

eral confluent whitish dime-sized nodules are seen beneath the pleura. Scattered throughout all the lobes a few subpleural pin-head sized whitish nodules are found.

Microscopically the consolidated lobe is seen to be atelectatic. The subpleural nodules are abscesses which contain a few streptothrix clumps and resemble those of rabbit No. 11. The pleura over the abscess is thickened by a tissue rich in spheroidal cells and leukocytes.

Smears made from masses around the trachea reveal a few rods with bipolar staining, and of the two broth tubes planted with large loopfuls of the material, one alone yields a delayed growth of the streptothrix.

The sections of the abscesses show a few streptothrix clumps without isolated rods. Other forms of bacteria were not seen. The remaining lobes contain smaller intrabronchial masses which surround the clumps, and there is a general catarrhal and productive bronchitis with nodular projections into and obliteration of the lumina of the tubes. The adventitia of the small blood-vessel is frequently infiltrated with spheroidal cells. The vascular changes otherwise resemble those described above.

SERIES III.—The following inoculations are for convenience grouped together.

Intraperitoneal injection: Rabbit No. 16.—April 20, injected into the peritoneum numerous granules of a streptothrix broth culture (15 days' growth). Rabbit killed 25 days later. Over site of injection, attached by a pedicle, is a pea-sized mass, hanging freely in the peritoneal cavity. Six small yellowish nodules adhere to the mesentery of the stomach and to the liver. The mesenteric lymph nodes are not affected. A yellowish-white, small, almond-sized mass is attached to the cæcum by fresh adhesions. On section the larger masses are made up of several confluent nodules, each surrounded by dense fibrous tissue. Smears made from the material reveal a few bipolar-staining rods. Small bits of the material were planted in four broth tubes, of which two after two weeks at 37° C. develop the clumpy variety of streptothrix culture.

The yellowish nodules forming the pendulous mass consist of a central collection of fragmented faintly-staining nuclei and necrotic granular material with a few clumps and clusters of streptothrix surrounded by a zone of vascular connective tissue, composed of spheroidal and larger polyhedral cells and fibroblasts, with eosinophilic granulations. The nodules on the serosa of the liver are of similar structure, the parenchyma of the liver being unaffected. Some of the nodules, however, are formed exclusively of vascular granulation tissue, and represent the nearly healed lesions. (Compare the result in this case with that in rabbits Nos. 1 and 2, in which the process of healing is complete, p. 181.)

Subcutaneous injection: Rabbit No. 17.—Feb. 25, injected subcutaneously the granules of an early broth culture. Twenty-four days later a small almond-sized nodule formed near the site of injection was removed. Coverslips revealed no organisms and no cultures were made. The mass on cross section is yellowish in color, resembles inspissated pus and is encapsulated, with a radially striated border of fibrous tissue.

In the sections stained by Weigert and by Sterling's gentian violet, a few clusters of streptothrix are found. The rods are similar to those met in the sections of the human lungs. The central part of the mass consists of a collection of leukocytes and necrotic material surrounded by vascular granulation tissue, which becomes densely fibrous at the periphery.

Intraperitoneal inoculations into guinea-pigs.—Three guinea-pigs were injected with large quantities of streptothrix culture obtained from various sugar-broth tubes on May 13.

The smallest animal became emaciated and died 13 days later. A few pin-head sized whitish nodules were found loosely attached to the mesentery. Smears reveal a few rods with polar staining; the inguinal lymph nodes were slightly swollen. The second animal was killed two weeks after inoculation. Negative autopsy. The third died 18 days after inoculation. Marked emaciation. A few small white nodules in the peritoneum similar to those found in the first guinea-pig. No cultures were made. No conclusions can be drawn from these inoculations in guinea-pigs, as they were not repeated.

SUMMARY OF THE EXPERIMENTAL LESIONS PRODUCED BY INOCULATIONS OF PURE CULTURES OF STREPTOTHRIX.—The streptothrix is pathogenic to rabbits and guinea-pigs, but death rarely supervenes from its action. In the rabbit subcutaneous injections and the escape of broth cultures into the peritracheal tissues, and intraperitoneal injections in rabbits and guinea-pigs produce local death of tissue and abscesses encapsulated by extensive zones of granulation tissue. In the lesions a few clusters of well-stained filaments or rods may occasionally remain, but individual rods are rarely found. The few clumps which are present in all the lesions represent, we believe, the discoid granules of the broth culture, which have undergone a peculiar transformation.

Introduction of pure cultures into the lungs through the trachea

is followed by similar phases of reaction on the part of the blood-vessels and of the tissue cells. When large quantities of the finely broken streptothrix granules of broth cultures are injected into the lungs, areas of consolidation composed of proliferated epithelial cells and leukocytes are formed, in addition to the intrabronchial masses of new-formed tissue, which encapsulate the clumps or discoid granules of the cultures. The largest intrabronchial masses in their early stages (two weeks after inoculation) resemble abscesses.

RESUMÉ.

The two cases of broncho-pneumonia in man forming the subject of this article were characterized by intense catarrhal and necrotic inflammation of the bronchi and by the presence of numerous streptothrix colonies in the bronchial lumina.

Introduction of the bronchial material of Case I into the trachea of three rabbits induced pulmonary abscesses and empyema of the pleura and pericardium in one of the animals.

Introduction of the bronchial material of Case II into the ear vein of a rabbit, and into the trachea of a second rabbit induced likewise pulmonary abscesses and empyema.

The empyemal pus of these rabbits contained filaments and rods morphologically identical with those composing the streptothrix colonies of the human cases.

From Cases I and II a streptococcus was cultivated on the ordinary media, the streptothrix not being isolated in culture directly from the human organs. By inoculating the fresh and sterile kidneys removed from a normal rabbit with the empyemal pus of a rabbit injected into the ear veins with the bronchial material of Case II the streptothrix from this case was finally isolated in pure culture, and its morphological and biological characters studied in detail.

COMPARISON WITH SPECIES PREVIOUSLY REPORTED.—Space does not permit a review of the records of the numerous species of the genus *Streptothrix* which have been found to bear a causal relation to various lesions or diseases, nor can we dwell upon the interesting rela-

tionships of the Streptothrices to a number of microorganisms which heretofore have been considered bacilli or fissure fungi.

For many years *Actinomyces bovis* Harz (*Streptothrix actinomyces* Rossi Doria) was considered to be the only representative of a genus of microorganisms to which the name "streptothrix" is at present generally applied.⁷ The growth flourished best under aërobic conditions and consisted of a felted network of branching mycelia mixed with shorter coccus- or spore-like forms. Inoculations of the cultures in animals were regularly negative.

Wolff and Israel in 1889 cultivated from two typical cases of actinomycosis a different species, *Streptothrix Israeli*, which flourished best under anaërobic conditions. In contrast to the morphology of the aërobic species the growth consisted mainly of rods with a few branching filaments, except in egg cultures where branching masses of mycelia developed. It grew only at incubator temperature, whereas the aërobic species developed equally well at room temperature. Typical actinomycotic lesions with rosettes and terminal bulbs followed introduction of the cultures into the peritoneum of rabbits.

Wolff and Israel's observations were not confirmed until A. Aschoff in 1895 cultivated a species similar to *Streptothrix Israeli* from a case of pulmonary actinomycosis.

Recently E. Levy has reported five cases of human actinomycosis from which he cultivated a strictly anaërobic species, which closely resembled *Streptothrix Israeli*.

Actinomyces bovis and *Streptothrix Israeli* are now considered to be two distinct species of streptothrix. The failure of Lucas, working in Levy's laboratory, to transform the aërobic species (*Streptothrix actinomyces*) into the anaërobic variety, with the morphological characteristics of *Streptothrix Israeli*, by cultivation through many generations under anaërobic conditions of growth, speaks against the identity of the two microorganisms. Levy does not favor the view held by some that *Streptothrix Israeli* is a pleomorphic bacillus.

⁷ Inasmuch as the name "streptothrix" had been previously appropriated for one of the hyphomycetes by Corda in 1839, it has been proposed by Levy to designate the group now generally called "streptothrix" as "actinomyces." We have, however, followed in this article the more usual designation at present.

Hugo Bruns has isolated what he considers to be a transitional form between the two species. In its cultural and morphological characteristics it closely resembles *Streptothrix Israeli*, but differs from it, in that it grows best aërobically.

The species isolated by us resembles closely in its morphological and cultural characteristics *Streptothrix Israeli*; but it is decidedly less pathogenic to animals. We do not consider the anaërobic preferences of *Streptothrix Israeli* sufficiently marked to form a point of differentiation between it and the species isolated by us, or by Bruns, since Wolff and Israel mention successful primary cultivation of their streptothrix from their first case on slant agar.

On account of the resemblance of Bruns's agar cultures to those of the tubercle bacillus it cannot be identified with ours, and the same objection presents itself in regard to the streptothrix isolated by Foulerton which he considers resembles the species isolated by Bruns.

Berestneff has isolated five species of streptothrix from human cases and although one of his species resembles ours in regard to its morphology and its broth cultures, we do not consider his descriptions sufficiently complete for purposes of species identification.

Paul Kruse cultivated from the yellowish granules in the pus of a maxillary tumor in a diabetic barber, a streptothrix which he considers closely related to or identical with *Streptothrix Israeli*. Except for the formation of acid in milk and broth cultures our species does not differ from Kruse's as far as one can judge from his descriptions.

Our streptothrix thus would seem to be, if not identical with, at least closely related to *Streptothrix Israeli*, and to the species isolated by Kruse. Whether it can be considered a variety or transitional form of Levy's anaërobic species must be left undecided.

The pulmonary lesions of the cases, however, do not resemble the broncho-pneumonic type of pulmonary actinomycosis described in the literature. The intense necrotic inflammation of the bronchi of the two cases reported, distinguishes them from the heretofore recorded cases of pulmonary actinomycosis or pseudo-actinomycosis.

We desire to express our thanks to Dr. A. Seibert and to Professor Delafield for the privilege of reporting these cases, and to Dr. Eugene Hodenpyl, Pathologist to the Roosevelt Hospital, for the opportunity of studying Case II.

It gives us great pleasure to acknowledge our deep sense of obligation to Prof. T. Mitchell Prudden for the kind assistance and suggestions which have always been at our disposal.

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DESCRIPTION OF PLATES XI-XVI.

PLATE XI.

Fig. 1.—Rod-like forms of streptothrix of Case II, from 2 days' growth of 3d generation on rabbit's kidney. Gram's stain.

Fig. 2.—Smear from colony, 7 days' growth, on glycerin agar, stained with methylene blue. Shows beaded filaments, small rods, and coccus-like forms. Compare with Fig. 3.

Fig. 3.—Smear from same colony as Fig. 2, stained by Gram. Note the differences in morphological appearances brought out by the two stains used for Fig. 2 and Fig. 3.

Fig. 4.—Coverslip from 3 days' growth in sugar broth. P. Ernst's stain. Shows branching forms and deeply stained granules.

Fig. 5.—Photograph showing streptothrix colonies growing on rabbit's kidney.

PLATE XII.

Fig. 6.—Photograph of section of rabbit's lung (Rabbit 3806) showing pneumonia and cavity produced by streptothrix infection following intraperitoneal inoculation of empyemal pus of infected rabbit.

Fig. 7.—Areas of infarction and abscesses in lung of the same rabbit (Rabbit 3806).

Fig. 8.—Scattered foci of consolidation in lung of rabbit killed 20 days after intratracheal inoculation with broth culture of streptothrix (Rabbit No. 11). With higher power these foci show intrabronchial masses of connective tissue growing around streptothrix clumps.

PLATE XIII.

A. Rods, filaments and curious forms of streptothrix seen in sections of bronchi in the human cases. They are stained reddish brown by Gram's method, this reaction being due to iodine.

B. Streptothrix forms from 10 days' growth of 14th generation in bouillon stained red in hot carbohc fuchsin, the intense color being retained after decolorization of the specimen in 97% alcohol.

PLATE XIV.

Streptothrix colony surrounded by cells and organizing tissue in rabbit's lung. The bulbous ends of the filaments are deeply stained by Weigert's fibrin dye.

PLATE XV.

Obliteration of rabbit's bronchus by new growth of connective tissue, the plug being channeled with canals lined by bronchial epithelium. The new tissue appears edematous. The section is from the lung of Rabbit No. 10, killed 37 days after intratracheal injection of broth culture of streptothrix (p. 183).

PLATE XVI.

Section of lung of Case I showing necrotic and suppurative bronchitis, with streptothrix clumps within the necrotic material in a bronchus. Fibrin and cells in the surrounding alveoli.

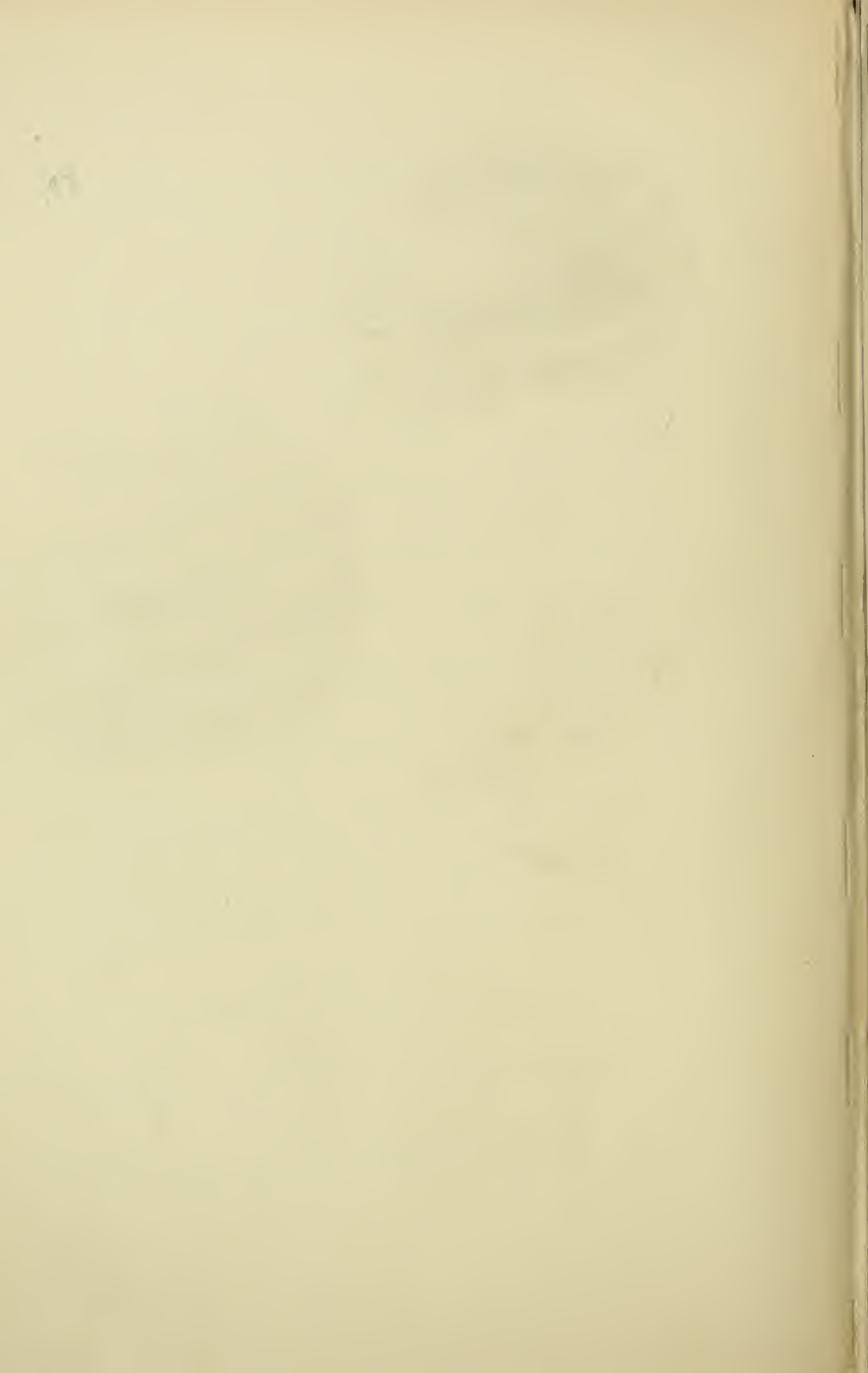




FIG. 5.

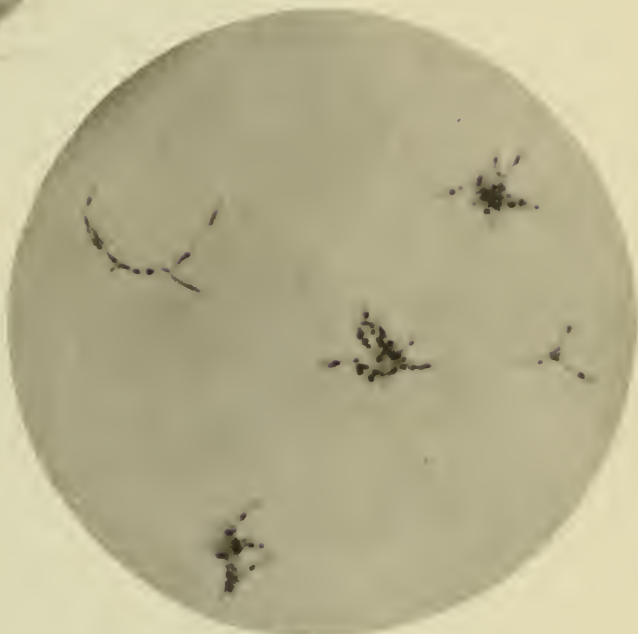


FIG. 4.

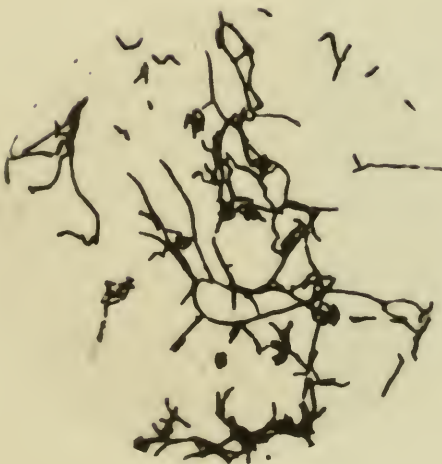


FIG. 3.

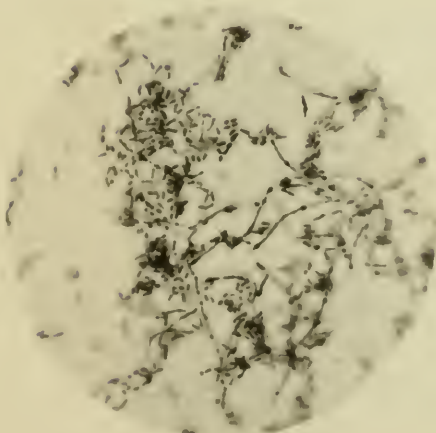


FIG. 2.



FIG. 1.

124



FIG. 8.



FIG. 7.

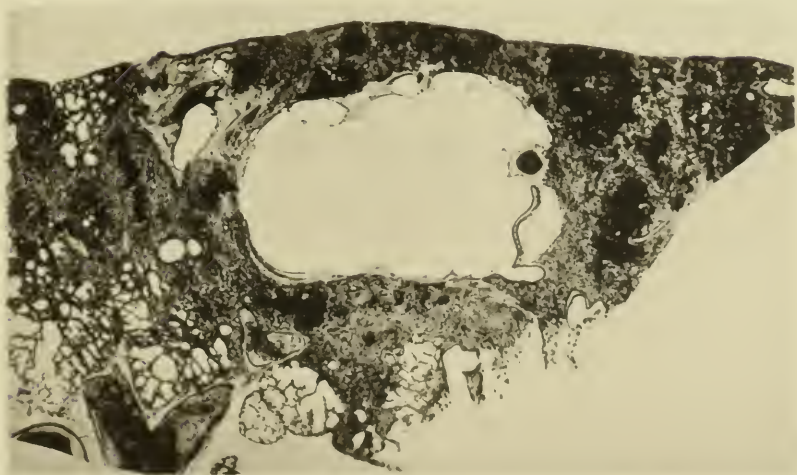


FIG. 6.

A



B.









ACUTE INTERNAL HYDROCEPHALUS. A CLINICAL AND PATHOLOGICAL STUDY.¹

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PLATES XVII AND XVIII.

Clinical History.—W. S., male, white, 33 years old, was admitted to the Philadelphia Hospital on April 15, 1899 complaining of great headache, general weakness and abdominal pain. His family and personal histories were unimportant, possibly except that four years before he had had typhoid fever. On April 13 he stopped work, complaining of violent headache and pain in the back of the neck. The pain continued throughout the night, and the following day he became delirious and was sent to the hospital.

Examination showed a muscular man with flushed face. He lay in bed with the head retracted and the legs strongly flexed upon the abdomen. The temperature was 102° F., pulse 82, and respiration 24. He was stuporous but could be roused by sharp questioning. He would answer a few questions properly and would then become incoherent. Physical examination revealed no abdominal or thoracic disease. There were no palsies. The head was in extreme extension and could not be flexed. Lateral movement of the head was also impossible on account of muscular rigidity. Attempts at passive movement caused severe pain. There was no muscular rigidity elsewhere. Sensation, so far as could be determined, was normal. He was too weak to stand. The tongue was brown, the lips cracked and the abdomen distended. The plantar jerks and knee jerks were absent even with reinforcement. The bladder and rectum were under control. The urine contained a trace of albumin, a few hyaline casts, and had a specific gravity of 1027. The red blood-corpuscles numbered 4,850,000, the white corpuscles 9000, and the hæmoglobin reached 75%. Widal's test was made repeatedly with

¹ Read at the meeting of the Philadelphia Neurological Society, December, 1899.

negative results. Various culture media inoculated with blood remained sterile. The tentative diagnosis was acute meningitis.

On April 22 Kernig's sign was present. Herpes of the lips and nose and a bluish mottling of the skin appeared. The mental state varied greatly. Sometimes the patient was restless and indeed quite violently delirious, at others stuporous, and again he would be almost normal, lying quiet, talking coherently and complaining only of headache and pain in the back of the neck. The pupils were dilated and slight lateral nystagmus was present. Slight deafness appeared. The plantar reflexes returned but the knee jerks remained absent. The eye grounds, examined by Dr. C. A. Oliver, were normal. On May 10 he was stone deaf. For several days he hiccoughed violently. The temperature which ever since his admission had been fluctuating between 101° and 103.4° F. now gradually dropped to normal and he improved greatly in all ways. Pain disappeared and the mind became clear and active. The knee jerks returned. Slight rigidity of the muscles of the neck persisted. His mental state was peculiar. He was perfectly coherent, could talk well and understood all that was said to him, but he was too well content with himself and his surroundings, he had no realization of his being sick and, though really too weak to stand, maintained that he was very well and would be out of doors in a day or two. He was trifling and jocose and, as his sister said, altogether unlike his usual self. He reminded one of a man in the beginning stage of parietic dementia. He gained greatly physically for a month and then the fever rose to 103° and the picture became the same as it was on admission. After a few days the fever again fell, and he again improved but for only a short time. On June 6 the temperature suddenly rose, the old symptoms returned, subsultus was added, nystagmus became marked and on June 9 he died.

Necropsy.—This was made the next day. The calvarium was normal. The dura was tense and elastic. The convolutions were broad, pale and flattened and the sulci were almost obliterated, appearing as lines beneath the pia. About the base over the cerebellum the pia was a little milky. The infundibulum was distended and pressed upon the optic nerves but not enough to distort them. On opening the ventricles a large amount of clear fluid escaped. All were dilated and the aqueduct of Sylvius was much wider than normal. The ependyma was boggy and separated easily from the underlying brain tissue. In the lateral ventricles it was roughened and at the tip of either inferior horn a band of white tissue stretched from the outer to the inner wall. The lining of

the fourth ventricle was somewhat boggy. The choroid plexus of the third ventricle was injected and oedematous, that of the lateral ventricles was rolled up into an oval mass the size of a hazel nut and adhered to the walls. The spinal cord was very wet and in the lumbar region were several small hernia-like protrusions of the white matter into the pia. The dura was a little thickened in the cervical region. The heart and lungs were normal. The right pleura was adherent. The spleen was cirrhotic and the kidneys showed a subacute parenchymatous nephritis.

Histological Examination.—On microscopic examination the choroid plexus of the lateral ventricles showed a small tumor-like mass which on cross-section consisted of a capsule surrounding meshes of blood-vessels. There were a large number of hyaloid bodies (Plate XVII, Fig. 2, A, E, B), often twenty in one field, staining deeply with hæmatoxylin but taking only a faint stain with Lugol's solution. There was marked infiltration of small round cells in the capsule (Plate XVII, Fig. 2, C) and foci of them scattered through the sections. Their nuclei stained deeply and were about half the size of those of the ependymal cells. The vessels were distended with red corpuscles. The choroid plexus of the third ventricle showed similar but slighter changes; that of the fourth was normal.

The ependyma of the third ventricle showed microscopically the same tendency to separate from the underlying tissue as was noted in the gross specimen. A marked ependymal proliferation was present in the lateral ventricles (Plate XVII, Fig. 1). The nuclei of the cells varied in size, shape, and staining properties. The ventricular surface was covered by an amorphous granular layer several times thicker than the ependyma and containing nuclei scattered through it. A golden yellow pigment was present in the ependyma of all the ventricles. The vessels beneath the ependyma to a depth of one-half centimetre were actively congested and were surrounded by round cells (Plate XVII, Fig. 1, D). These cells not only occupied the perivascular spaces but also extended some distance into the surrounding tissue. The greater number of them were small round cells with nuclei slightly smaller than the nuclei of glia cells and surrounded by a narrow ring of cell substance. Though the nuclei of the glia cells within the area of infiltration were swollen and possibly proliferating we feel confident that the larger number of these cells did not arise from the glia. Scattered among the smaller nuclei larger, faintly-staining nuclei resembling very closely those of the endothelial cells of the capillaries were occasionally seen. They were probably endothelial in origin but of this there is no direct evidence. No

polynucleated cells were present in the infiltration. Some of the large pale nuclei were irregular in outline but did not resemble the nuclei of polynuclear leucocytes in any other respect.

The neuroglial cells also showed proliferative changes. The nuclei of those immediately beneath the ependyma were at least twice the normal size and were surrounded by distinct cell bodies from which the processes radiated (spider cells). Further from the surface the nuclei became smaller, the protoplasm less, and the radiating processes finer until at a depth of about three quarters of a centimetre the tissue became normal. The area of change in the glia cells corresponded closely to that of congestion and round-cell infiltration.

The nature of the lesions suggested that they might have been caused by the reaction of the tissues to some toxic or irritative constituent of the ventricular fluid.

The cortical and spinal ganglion cells stained by Nissl's and Weigert's methods and by carmine showed nothing abnormal. The cranial nerves were stained by the Marchi method and carmine. The fourth and sixth showed a few black granules. The eighth pair were very much degenerated; the others normal. The spinal nerve roots showed only a few black granules in the lumbar posterior roots. The white matter of the cord showed no degeneration by the Marchi, Weigert, and carmine stains.

To sum up: A man is suddenly seized with fever, bradycardia, constipation, rigidity of the muscles of the neck, headache, stupor and delirium. After three weeks, during which the intensity of the symptoms varies greatly, he improves very much physically but shows many of the mental symptoms of parietic dementia. A week later fever and the meningeal symptoms return, last about a week, again intermit for four days only to return again and end in death. Post-mortem examination reveals only a moderate internal hydrocephalus, proliferation of the ependyma and ependymal glia, perivascular round-cell infiltration in the sub-ependymal tissues, and sclerotic and degenerative changes in the choroid plexus. What caused the lesions?

Internal hydrocephalus from mechanical causes is quite common, but idiopathic internal hydrocephalus is, or seems to be, rare and has attracted the attention of clinicians and pathologists only in recent years. Before the discovery of the tubercle bacillus it was quite

common to call cases of hydrocephalus tubercular even though tubercles were not present. Barthez and Rilliet² separated these from those manifestly tubercular, but the credit of having established idiopathic internal hydrocephalus as a distinct clinical and pathological entity belongs to Quinke.³ There are two varieties, one acute, the other chronic but often having acute exacerbations.

The acute variety is most frequent in children. In adults it usually follows injury to the head, the infectious fevers, especially typhoid and pneumonia, and acute or chronic alcoholic poisoning. The onset is sudden with headache, delirium, photophobia, vomiting and retraction of the head—symptoms resembling the irritative stage of septic or tubercular meningitis from which affections it may be impossible to differentiate it. Fever, however, may be absent or not so high as in septic meningitis and may either quickly disappear or vacillate with the other symptoms. The headache and muscular rigidity are not so intense nor so constant and the delirium, instead of persisting, alternates with periods of normal consciousness. The intermittency of the symptoms and the early increase of intracranial pressure, shown by choked disc, paralytic mydriasis, and, in early life, enlargement of the head, are characteristic. Examination of the cerebrospinal fluid is important. In tubercular meningitis the bacillus is often, although not always, present in the fluid withdrawn by lumbar puncture, and in septic meningitis inoculations of culture media give characteristic growths, and the fluid is either turbid or distinctly purulent and contains endothelial cells, leucocytes, red corpuscles, and pus microorganisms. In idiopathic hydrocephalus the fluid is under higher tension than normal varying from 150 to 700 mm. (water manometer). It differs but slightly in its constituents from the normal, is clear and transparent, with a specific gravity of 1008 and contains albumin and sugar (?) in small quantities.

The chronic variety often follows the subsidence of the acute symptoms or, in children, may appear only with the increase in intra-

² Barthez and Rilliet. *Traité clinique et pratique des maladies des enfants*. Paris, 1861.

³ Quinke, Volkmann's *Samml. klin. Vortr.*, 1893, n. F., No. 67, and *Deutsche Zeitschr. f. Nervenheilk.*, 1896, ix, p. 149.

cranial pressure caused by the union of the bones of the skull. A receding optic neuritis may be the only clinical manifestation. In other cases the symptoms may for a long time be vague and inconstant so that mere neurasthenia may be suspected. A large group present the symptoms of brain tumor. Optic neuritis is almost constant and transient or permanent bitemporal hemianopsia caused by varying pressure of the distended infundibulum on the optic chiasm may be present. Headache, vomiting, vertigo and local or general convulsions, and cranial nerve palsies are frequent. Localizing symptoms are almost never present except in the rare cases in which the hydrocephalus is confined almost entirely to the fourth ventricle and causes symptoms of cerebellar disease. The long course of the disease often ending favorably or seeming to do so only to recur, and the good effect of lumbar puncture point to the true nature of the disease, especially if the spinal fluid is found to be under high pressure at the time of puncture.

Our case in the early stages was diagnosed as a meningitis of the convexity and of the cord. Typhoid and spotted fever were both thought of but the continuous absence of the Widal reaction, the absence of spots, the non-enlargement of the spleen, and the clinical course excluded the former and the absence of eruption, of leucocytosis, of spinal-root symptoms, and the negative result of bacteriological investigation made the latter impossible. The peculiar variability of the symptoms, the afebrile intermissions, and the curious mental state made the diagnosis doubtful but it was retained as the best working hypothesis.

There are many points of clinical interest in the case. Kernig's sign which was at one time thought to be pathognomonic of meningitis was present during almost the whole course of the disease. The absence and subsequent return of the knee jerk and plantar reflex is also interesting. Absence of the knee jerk is not very uncommon in brain tumor but its return after an absence of three weeks without any lesion in the cord to explain it must be very rare. The absence could not have been caused by œdema of the cord because at autopsy that was very great though the reflex had returned several weeks before. We can only note the phenomenon, not explain it.

Central deafness with degeneration of both eighth nerves is not mentioned in any other case. It probably was caused by an unusual distribution of the pressure from the ventricles on the nerves at their exits. The optic nerves are most frequently affected and after them the sixth and seventh. In our case the optic nerves were normal save for a small focus of old degeneration near the centre.

The mental state may resemble parietic dementia. Indeed in Prince's⁴ cases so close was the resemblance that that diagnosis was held for a time. Jocosity, a total neglect of the relative importance of things, and mild delusions of grandeur were the predominant factors in the mental attitude of our patient.

The pathological findings are interesting because in only a few of the cases heretofore reported have changes in the ependyma and subependymal tissues been described. Quincke⁵ explains the absence of inflammatory changes in his cases by the subsidence of the primary hyperæmia subsequent to the occurrence of the hydrocephalus. The membranes of the brain may according to him be the seat of an acute serous inflammation like that which occurs in the pleura and the synovial linings of joints. In other cases the acute onset of the symptoms and their rapid disappearance make it seem probable to him that changes similar to those of angio-neurotic œdema may occur. In our case there were both recent and old changes in the choroid plexus. The proliferation of the interstitial tissue, the dilatation of the vessels, the round-cell infiltration, and the ependymal changes all pointed to an acute inflammation. The encapsulation of the plexus, the adhesions to the ventricular wall, and the bands across the tips of the ventricles indicated an old process. The large number of hyaloid bodies in the plexus of so young a man is evidence of degenerative change in the vessels leading to their obliteration and subsequent change into hyaloid material. All the steps in the process from hyaline degeneration of the media to complete obliteration, fragmentation, and calcification of the fragments are fairly well shown in the sections. That these bodies may have another and very

⁴ Prince. *Journal of Nervous and Mental Disease*, 1897, xxiv, p. 473.

⁵ Op. cit.

different origin, namely from proliferated endothelial cells blocking up the lymph spaces and undergoing hyaline degeneration, there is abundant evidence in the same sections, but the changes in the blood-vessels are the more important in connection with the other evidences of a chronic and acute inflammation in the plexus.

The changes in the ependyma correspond to those in the choroid plexus. The thickened ependymal membrane and the adhesions and roughened surfaces of the lateral ventricles were of long standing. The acute inflammatory changes were widespread. Some authorities⁶ hold that since the ependyma consists only of a layer of cells without blood-vessels it cannot be the seat of acute inflammation, but the distended network of capillaries immediately beneath the ependymal layer, the perivascular round-cell infiltration, the amorphous exudate on the surface and the hypertrophic changes in the glia must be considered to prove the inflammatory nature of the process. The fact that these changes were confined almost wholly to the ependyma and extended only a few millimetres beneath, gives the impression that they were the results of a local reaction of the cerebral tissues to some toxic action of the ventricular fluid rather than an acute primary inflammation leading to a serous exudation. To determine if possible which of these two conditions was present and to discover the effect of toxins and acids on the ependymal membranes we made the following experiments:

Sterilized urine, glycerine extract of the adrenals (P. D. & Co.), tuberculin, hydrochloric and carbolic acid were injected into the ventricles of kittens by means of a large hypodermic syringe.

The three adrenal kittens died within twenty-four hours. A slight increase of the nuclei surrounding the vessels immediately beneath the ependyma and dilatation of the capillaries of the brain substance for a short distance were the only changes noted. In one kitten there was intense dilatation of the vessels of the choroid plexus with hæmorrhages into its meshes.

Three kittens injected with urine were killed after three, six, and ten days. The changes were the same in all, differing only in intensity

⁶ Boenninghaus, *Die Meningitis serosa acuta. Eine kritische Studie.* Wiesbaden, 1897.

(Plate XVIII, Fig. 4). They consisted of a proliferation of the ependyma with an amorphous exudate upon the surface, swelling of the glia fibres which stained deeply, and a perivascular small round-cell infiltration, the whole resembling very much the condition found in the case here reported. In one specimen the layer of cells covering the choroid plexus failed to stain and looked very much like a fatty reticulum. In this specimen also a layer of round nuclei occupied the space immediately beneath the ependyma. Tuberculin gave similar but less marked results.

As dilute hydrochloric and carbolie acids produced the same effect we will describe the former only. The animals were killed at the end of a week. There was no excess of ventricular fluid. The ependyma looked normal and was not boggy or roughened. On microscopic examination (Plate XVIII, Fig. 3) the ependymal layer was intact, but the cells stained very faintly, the nuclei could hardly be seen, and the cell bodies were granular and their margins indistinct. Where this condition was most intense a granular layer covered the ependyma, evidently the debris of degenerated cells. Immediately beneath the ependyma and most marked on the under and inner surfaces of the ventricles a layer of round nucleated cells was present. They were four and five deep in certain areas and in others suddenly disappeared. They had small, round nuclei which stained deeply and were of the same size and character as those surrounding the deeper vessels. No polynuclear cells were found among them. Their probable origin was the network of capillary vessels beneath the ependyma. Columns of small nuclei surrounding the smaller vessels extended from this layer deeper into the brain substance. The glia network within the zone of capillary dilatation and perivascular infiltration was close meshed and had the appearance of a fibrillar structure running parallel to the ependymal surface. The columnar cells of the choroid villi were granular. The superficial cells were mere shadows and often the outer border was absent making the surface of the plexus look frayed. The nuclei were either absent or only outlined and failed to stain. The condition appeared to be caused by a proliferation of the cells: the outer layer becoming degenerated and finally breaking down into an amorphous mass resting upon the surface of the other cells.

We conclude from these experiments that the non-purulent inflammation of the ependyma produced by acid irritants differs only in degree from the reactive changes following the injection of toxins into the ventricles. Changes in the ependyma without changes in

the subjacent tissue probably do not occur. The inflammatory condition experimentally produced, by whatever agent, did not cause any increase in the ventricular fluid and the only evidence of an exudate from the ependyma was the amorphous material which probably was made up of degenerated cells.

The microscopic sections in the toxine experiments resembled the sections from the case reported and to that extent confirm the opinion that the changes found were secondary to a toxic condition of the ventricular fluid. The clinical history offers other evidence in support of this view. For example the mental condition of the patient, corresponding to that seen in other auto-intoxications, would be best explained by a such an hypothesis. The exacerbations which Quinke compares in their sudden development and variability to angio-neurotic œdema appear to us to be rather the manifestations of varying intensity of auto-intoxication, such as occurs in uræmia and syphilis. Finally the hydrocephalus alone by its mere mechanical action, if sufficient fluid is present, can cause many symptoms.

DESCRIPTION OF PLATES XVII AND XVIII.

PLATE XVII.

FIG. 1. Section of the floor of the lateral ventricle. Stained with hæmatoxylin-eosin. The marked irregularity of the floor is shown—caused partly by the folding of the ependyma (*C*), partly by the amorphous exudate on the surface (*B*), and partly by the hypertrophy of the sub-ependymal glia (*A*). The zone of perivascular small, round-cell infiltration (*D*) is seen extending some distance beneath the ventricular surface.

FIG. 2. Section of the choroid plexus of the lateral ventricle. *C*, the capsule very rich in nuclei. Hyaloid bodies in different stages are seen at *A*, where the hyaline change with calcification is beginning in a vessel, and at *B* and *E* has advanced to irregular hyaloid forms. The vessels of the plexus contain many leucocytes which are very rich in a granular pigment (*D*).

PLATE XVIII.

FIG. 3. Showing the reactive changes in the ependyma and choroid plexus of a cat after the injection of hydrochloric acid (5%) into the ventricle. *A*. Granular degeneration of ependymal cells. *B*. Layer of round nuclei immediately beneath the ependyma. *C*. Perivascular infiltration of round cells extending deeper into the brain substance.

FIG. 4. Ventricular surface of the brain of a cat after injection of sterile urine. *A*. Granular degeneration of ependyma cells. *B*. Sub-ependymal layer of round nucleated cells. *C*. A vessel surrounded by an accumulation of round cells. The blood-vessels deeper in the tissue are not affected.

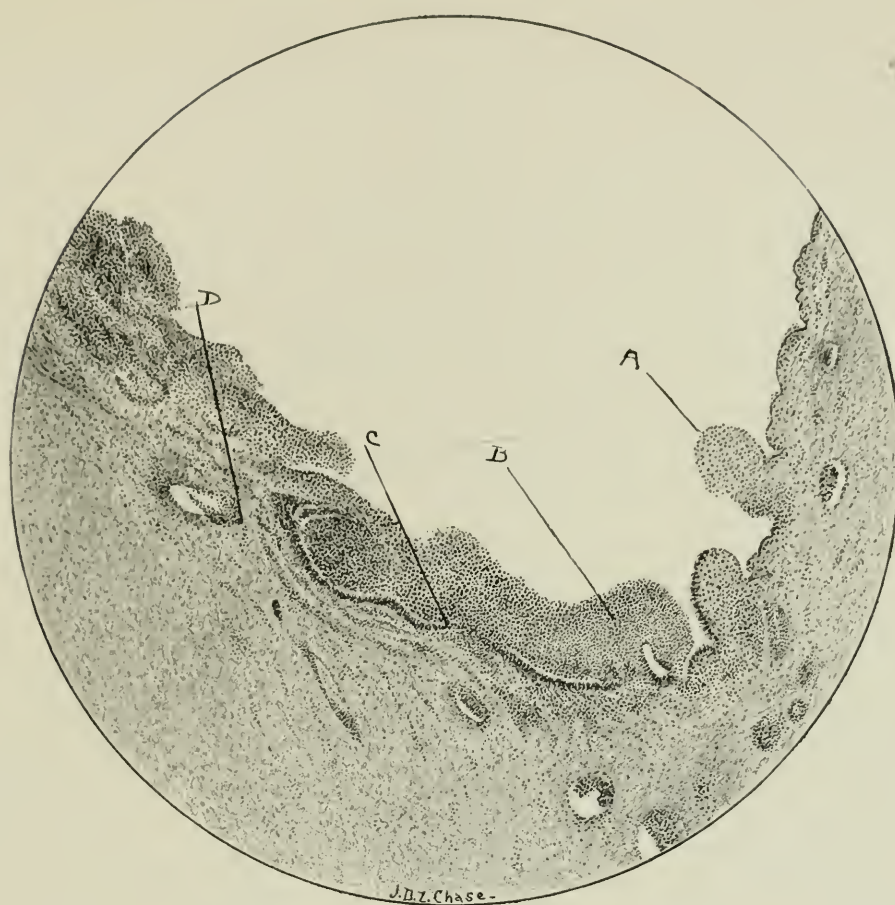
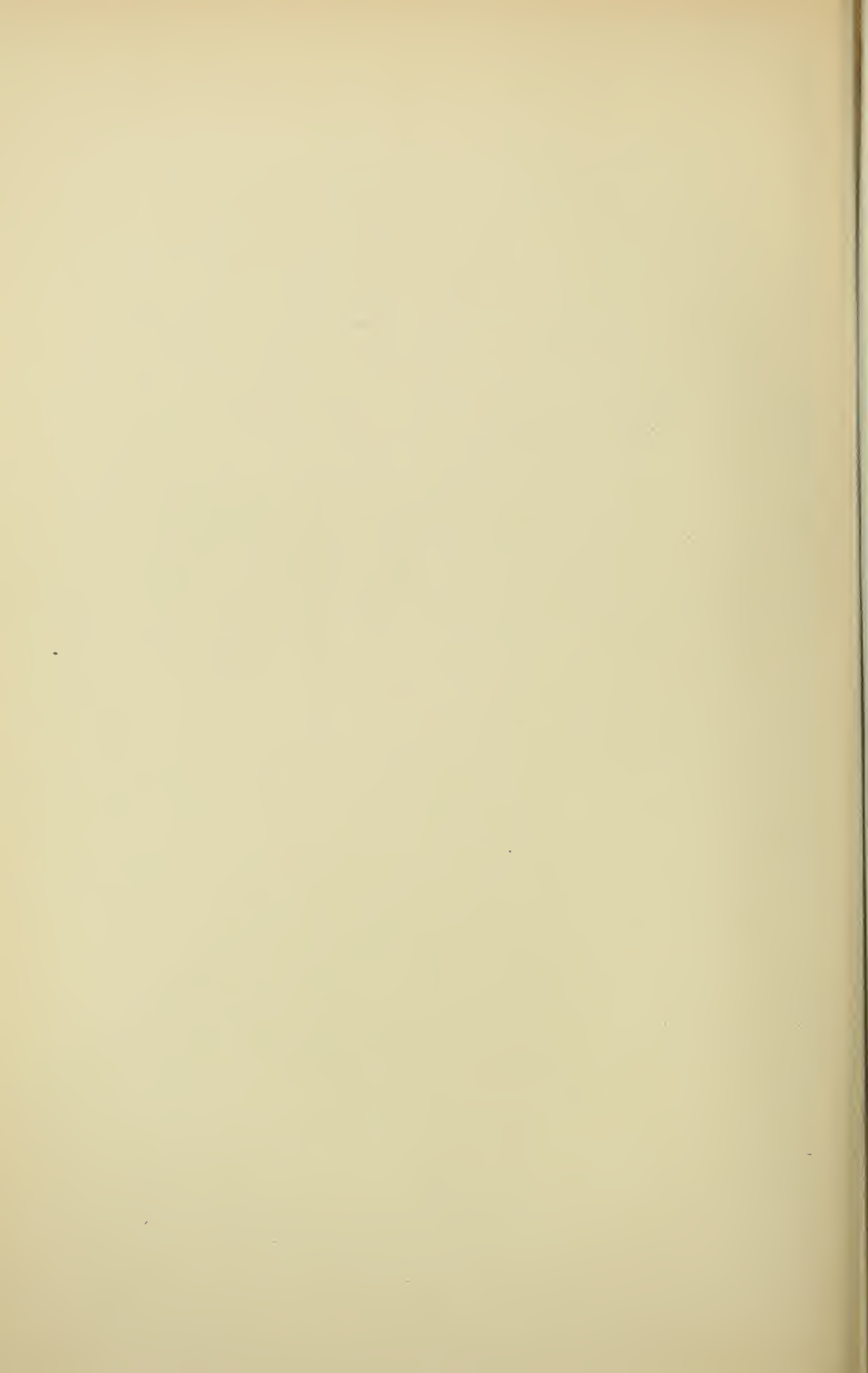


FIG. 1.



FIG. 2.



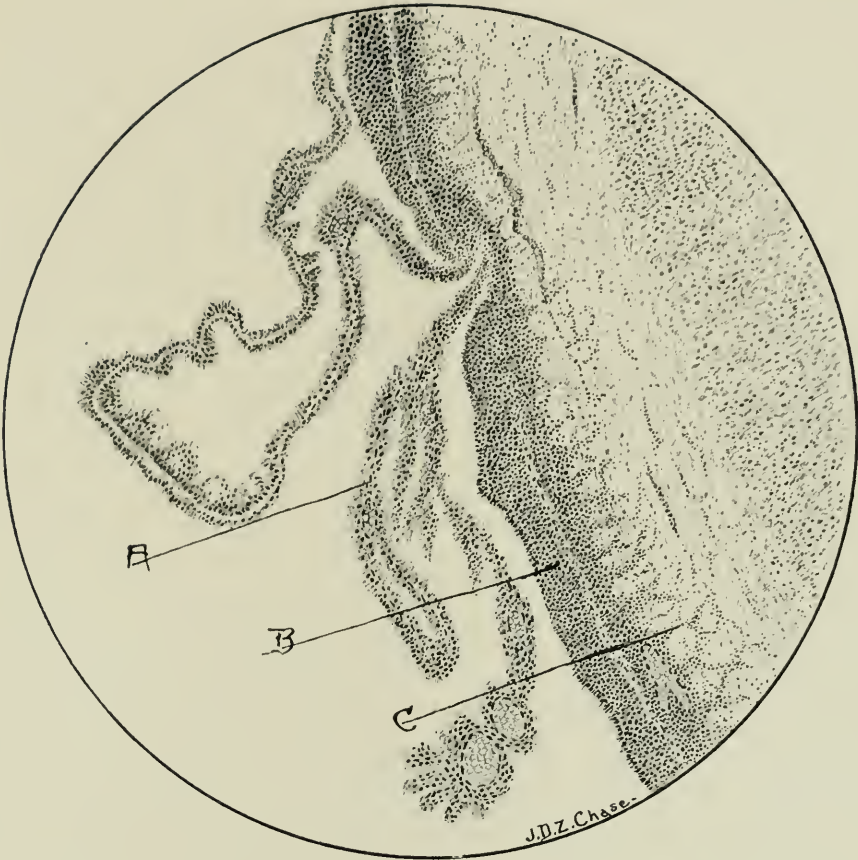


FIG. 3.



FIG. 4.

A PRELIMINARY REPORT ON ACID-RESISTING BACILLI, WITH SPECIAL REFERENCE TO THEIR OCCURRENCE IN THE LOWER ANIMALS.¹

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At the suggestion of Professor Dock, I began four years ago to make some investigations on the occurrence of the smegma bacillus in man. While pursuing this work, which is not yet completed, it occurred to me that it might be not only of interest but of importance to know whether the smegma bacillus or bacilli resembling those of tuberculosis existed to any great extent in the lower animals. It is this point I wish to consider here.

Resistance to decolorization by strong acids (Säurefestigkeit), as was first shown by Ehrlich, is the most characteristic staining reaction of the tubercle bacillus and, at the date of its discovery, differentiated this bacillus from all other known bacteria except the bacillus of leprosy. Since this time, however, a number of other acid-resisting bacilli have been discovered, the list increasing with especial rapidity during the last few years. Soon after the discovery of the tubercle bacillus in 1882, G. Zahn² noted the presence in non-tuberculous sputum of bacilli resembling the tubercle bacillus in staining reaction, and recently acid-resisting bacilli resembling tubercle bacilli have been found in the sputum in cases of pulmonary gangrene by A. Fraenkel,³ Pappenheim,⁴ and Rabino-witsch.⁵ Laabs⁶ and Moëller⁷ have also recorded the presence of acid-resisting bacilli in non-tuberculous buccal secretion and sputum.

¹ Read at the meeting of the Medical Society held in the Medical College, Ann Arbor, December 14, 1899.

² *Med. Cor.-Bl. d. württemb. ärztl. Vereins*, 1884, liv, p. 129.

³ *Berliner klin. Wochenschr.*, 1898, pp. 246 ; 880.

⁴ *Ibid*, 1898, p. 809.

⁵ *Deutsche med. Wochenschr.*, 1900, p. 257.

⁶ *Inaug.-Diss.*, Freiburg i. Br. 1894.

⁷ *Zeitschr. f. Hyg.*, 1899, xxxii, p. 211.

The prototype and longest known of these acid-resisting pseudo-tubercle bacilli is the smegma bacillus first described in 1885 by Alvarez and Tavel⁸ and by Matterstock⁹ and Bitter,¹⁰ and obtained in artificial culture by Laser¹¹ and by Czaplewski.¹² The occurrence of acid-resisting bacilli in the human intestine is mentioned by Crämer,¹³ de Giacomi,¹⁴ and von Jaksch,¹⁵ so that there is proof that bacilli resembling in tinctorial properties and often more or less closely in morphology the tubercle bacillus may appear in the secretions from the human genito-urinary, intestinal and respiratory tracts. The acid-resisting bacillus cultivated by Czaplewski¹⁶ from a case of leprosy is probably not identical with the bacillus of leprosy. Dietrich¹⁷ reports an interesting observation of the presence of acid-resisting bacilli resembling the tubercle bacillus in a suppurating ovarian cyst which had ruptured into the intestine, their presence, together with the symptoms, leading to the erroneous diagnosis during life of tuberculous peritonitis.

An especial incentive to the search for acid-resisting bacilli has been the detection by Petri and by Rabinowitsch of such bacilli in butter and by A. Moëller of similar bacilli in timothy grass and dung. Since the first demonstration of tubercle bacilli in butter by Brusaferro¹⁸ in 1890, this hygienically important subject has been investigated by several writers with most discordant results, the percentages of samples of butter in which the tubercle bacillus was found varying from 0 to 100. Obermüller¹⁹ examined 14 samples of butter from a single source in Berlin, all of which he claims contained the tubercle bacillus. Rabinowitsch,²⁰ who examined butter from a number of shops in Berlin and in Philadelphia, demonstrated the tubercle bacillus in all the samples from one of the largest Berlin houses, whereas all of the other samples were free from this bacillus. In a considerable proportion of the samples,

⁸ *Progrès méd.*, 1885, 2. s., ii, p. 135, and *Arch. de phys. norm. et path.*, 1885, 3. s., vi, p. 303.

⁹ *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1885, p. 65.

¹⁰ *Virehow's Archiv*, 1886, cvi, p. 209.

¹¹ *Münch. med. Wochenschr.*, 1897, p. 1191.

¹² *Ibid.*, 1897, p. 1192.

¹³ *Sitzungsb. d. phys.-med. Soc. zu Erlangen*, Dec. 1882.

¹⁴ *Fortschr. d. Med.*, 1883, i, p. 145.

¹⁵ *Klinische Diagnostik innerer Krankheiten*. Wien and Leipzig, 1896.

¹⁶ *Centralbl. f. Bakter.*, 1898, xxiii, pp. 97, 189.

¹⁷ *Berliner klin. Wochenschr.*, 1899, p. 189.

¹⁸ *Baumgarten's Jahresbericht*, 1890, vi, p. 271.

¹⁹ *Hyg. Rundschau*, 1897, vii, p. 712.

²⁰ *Zeitschr. f. Hyg.*, 1897, xxvi, p. 90, and *Deutsche med. Wochenschr.*, 1899, p. 5.

however, she found bacilli morphologically and tinctorially resembling the tubercle bacillus. Butter containing these bacilli, injected into the peritoneal cavity of guinea-pigs, produced nodules superficially like tubercles and containing the bacilli, but distinguishable from true tubercles by their histological characters. Injection of pure cultures of the bacilli did not produce tubercles. Rabinowitsch says that Koch in 1896 recognized the occurrence in butter of acid-resisting bacilli resembling the tubercle bacillus. Shortly before Rabinowitsch's description of these bacilli in butter, there appeared, as a comment upon Obermüller's disquieting observations, a brief statement concerning the detection of these pseudo-tubercle bacilli by Petri,²¹ who published in the following year²² a fuller description in his study of the question of tubercle bacilli in butter.

The publications of Rabinowitsch and of Petri have led to further investigations concerning the presence of acid-resisting bacilli resembling the tubercle bacillus in butter, among which may be mentioned those of Hormann and Morgenroth,²³ Grassberger,²⁴ Herbert,²⁵ Weissenfeld,²⁶ Ascher,²⁷ Coggi,²⁸ and Korn.²⁹ While the results vary as to the frequency of these bacilli in butter, some indeed being negative, and also to some extent as to their characters, it is established that bacilli morphologically and tinctorially like tubercle bacilli have been repeatedly found in butter, and their occurrence should lead to great care in the diagnosis of suspected tubercle bacilli in this material, as well as in milk. Culturally these bacilli can be readily distinguished from the tubercle bacillus, and the same distinction can usually be made without much difficulty in their pathogenic properties.

Grassberger inoculated the peritoneal cavity of 20 guinea-pigs with Vienna market butter and noticed in 10 of these characteristic anatomical changes. Firm whitish yellow masses were found on the peritoneum. These masses were made up of a delicate fibrinous frame-work with remnants of butter and large numbers of bacilli within. The bacilli stained red when treated with carbolic fuchsin and decolorized in the nitric acid

²¹ *Hyg. Rundschau*, 1897, vii, p. 811.

²² *Arch. u. d. k. Gesundheitsamte*, 1898, xiv, p. 1.

²³ *Hyg. Rundschau*, 1898, viii, pp. 217; 1081.

²⁴ *Münch. med. Wochenschr.*, 1899, Nos. 11 and 12.

²⁵ *Arch. u. d. path.-anat. Institut zu Tübingen*, 1899, iii, p. 207.

²⁶ *Berliner klin. Wochenschr.*, 1899, p. 1053.

²⁷ *Zeitschr. f. Hyg.*, 1899, xxxii, p. 329.

²⁸ *Giorn. d. R. Soc. Ital. d'igiene*, 1899, No. 7.

²⁹ *Arch. f. Hyg.*, 1899, xxxvi, p. 57, and *Centralbl. f. Bakt.*, 1899, xxv, p. 532.

solution. He obtained pure cultures of this so-called butter bacillus, but like Rabinowitsch he failed to produce lesions in guinea-pigs by their inoculation. If, however, he mixed the culture with sterilized butter or sterilized paraffin and inoculated the mixture into guinea-pigs, the animals died and on post-mortem examination showed the characteristic nodules described above. Sterilized butter or sterilized paraffin produced no lesions when inoculated. If preparations of this organism were left for 24 hours in alcohol and ether they no longer resisted the decolorizing solution. Grassberger found no genuine tubercle bacilli in the samples of butter examined. He further observed that if guinea-pigs were inoculated with Kretz's bacillus of timothy grass lesions similar to those produced by the butter bacillus occurred.

In her first publication on this subject Rabinowitsch stated that Capaldi in examining her specimens was impressed with the resemblance between these butter bacilli and acid-resisting bacilli which he had found in cow's dung. Severin³⁰ had previously reported the existence of such bacilli in horse's dung and Ferran³¹ in cow's, horse's and human feces. Of especial interest and importance are the recent observations of Alfred Moëller³² who has cultivated from vegetable fodder and from the dung of different animals bacilli which he designates respectively as the dung bacillus, the timothy bacillus or grass bacillus I, and the grass bacillus II. These bacilli of Moëller are even more resistant to decolorization by acids than the tubercle bacillus, which they also resemble morphologically, but are distinguished from the latter by cultural characteristics. Especially noteworthy are Moëller's statements concerning the production of nodules closely resembling genuine tubercles by inoculation into guinea-pigs of pure cultures of these bacilli. Moëller speaks of confirmation or control of his results by Czaplewski, Kretz, and Lubarsch.

There can be no doubt that different species or varieties are represented among the various acid-resisting bacilli described by the investigators cited as "smegma bacilli," "butter bacilli," "dung bacilli," "timothy" or "grass bacilli," etc., but further work is needed to elucidate the differences or relationships between the members of this interesting group of bacteria.

³⁰ *Centralbl. f. Bakter.*, Abth. II, 1895, i, p. 97.

³¹ Abst. in *Centralbl. f. Bakter.*, 1897, xxii, p. 484.

³² *Deutsche Med.-Ztg.*, 1898, p. 135; *Deutsche med. Wochenschr.*, 1898, p. 376; *Therap. Monatsh.*, 1898, xii, p. 607, and *Centralbl. f. Bakter.*, 1899, xxv, p. 369.

As already stated, the special object of my investigation was to determine to what extent acid-resisting bacilli are to be found on the bodies of animals, a subject which has received hitherto but little attention. The occurrence of such organisms in animal products was also considered, but to a less extent. Inoculation and culture experiments are under way and these results will be published later. In my work I adopt the name acid-resisting bacillus in preference to the too loosely used term smegma bacillus. It remains to be proved whether there is any relationship of the smegma bacillus to similar bacilli found on parts of the body other than the genitals, and to some of those found in animal products. Moreover the question has been raised as to whether the so-called smegma bacillus as it is seen in man, is a distinct species or whether the term represents a group of closely allied organisms.

The material for this investigation was obtained by scraping the parts with a blunt instrument. At times, as in the case of soft smegma, the material was easily obtained, and in that case a small bit was crushed between two cover glasses. In the case of dry epithelial scales, the scrapings were rubbed up with a drop or two of sterile water and with this suspension coverslip preparations were made and allowed to dry in an incubator. The slips in all cases were fixed by passing three times through the flame. They were all stained with hot carbolie fuchsin, after which they were thoroughly treated with fresh 25 per cent nitric acid solution, washed in water, and counter-stained in Loeffler's or aqueous methylene blue. From three to six slips were thus prepared from each specimen and examined. The instruments used were sterilized before each examination. Fifty-five animals and a few samples of milk and feces were examined.

Horse.—The smegma of the horse exists in large amount; 10 to 20 grammes, or more, may be obtained from the sheath at one time. It is of a grayish-black color and is tolerably firm. The smegma of the mare is quite soft, of a light gray color and is found in small amount. The bacilli found in horse's smegma are for the most part slender, clean-cut rods and resemble very much the tubercle bacillus. Other forms may at times predominate, thus in the scrapings

from the vulva of one mare there was found on one slip a large number of capsulated bacilli which remained red after decolorizing in acetic acid. The smegma of one horse, two mares, one Shetland horse and one Shetland mare were examined, with positive results in all.

Dog.—The smegma of the dog, in the cases examined, was a creamy, semi-fluid, yellowish substance, existing in small amount. In this substance bacilli precisely resembling the tubercle bacillus may be found. Many beautiful beaded organisms were seen in some preparations. In the yellowish discharge so commonly seen on the penis of dogs similar bacilli were seen. The combination of these bacilli with the many leucocytes, mucus, and other microorganisms which abound, make a picture difficult to distinguish from that of tuberculous sputum. In this discharge, which apparently comes from the urethra, numbers of large phagocytes may be seen, but without bacilli. Four dogs were examined with positive results in three, negative in one.

Cow.—The cows used for this experiment were those of a dairy farm. There was no evidence of external disease of the teats or udders; aside from an occasional wart, they were to all appearances normal. These cows had not been subjected to the tuberculin test. The material for the examination was obtained by scraping the moistened teat or udder with a scalpel. The scrapings—dust, epithelial cells, etc.—were allowed to fall on bits of moistened filter paper placed in small tin boxes; coverslip preparations were made as already described.

It was not difficult to demonstrate acid-resisting bacilli in these scrapings. Generally speaking, these bacilli were much thicker than the tubercle bacillus. In some slips several different forms may be seen, as bent rods, rods with irregular outline, beaded forms, and club-shaped bacilli. In one specimen there were a number of slender bacilli, sometimes slightly bent, which stained a distinct but light pink, some isolated and scattered through the field, others in groups of five or more. These bacilli precisely resemble the tubercle bacillus. On account of the large amount of epithelium which takes the carbolie fuchsin deeply and resists decolorizing solutions, special

care was taken to expose these specimens for a considerably longer time than is necessary for the decolorization of sputum. Some slips were left in the acid solution until no further trace of red was visible to the naked eye, and still in these preparations red bacilli could be demonstrated. The bacilli were usually seen free from the epithelial cells. In every cow examined large numbers of yeast cells and spores which stained red were found. The habit so prevalent of lubricating the hands of the milker with the first strippings undoubtedly accounts for the invariable presence of yeast cells. The sugar in the milk favors the growth of these organisms.

In all, eight cows were examined for acid-resisting bacilli with positive results in five, negative in three. The so-called glair or vaginal mucus of one cow gave a negative result.

Eight preparations were made from the sediment obtained from a centrifugal separator. Each specimen showed many acid-resisting bacilli, some of which might easily be mistaken for the tubercle bacillus.

Guinea-pig.—The material for this examination was obtained from the glans penis, prepuce, vulva, anus, and from the mammæ of both male and female. Although the prepuce is quite long and completely covers the glans, yet the latter as a rule is very dry and only at times can a slight collection of a yellowish white material or smegma be seen. This substance has an odor similar to that of human smegma. The vulva which has no deep folds gave material which looked like slight epithelial desquamation mixed with dirt. All told, five male and five female guinea-pigs were examined. In all the males, acid-resisting bacilli were found on the genitals. On the mammæ of one and on the anus of another similar organisms were demonstrated. All the females but one gave positive results. The mammæ of two of these were examined with positive results in one. The organisms usually found were shorter and thicker than the tubercle bacillus. Occasionally longer and more slender forms were seen on the mammæ. Not infrequently large groups of red bacilli can be seen on a faded pink epithelial cell.

Rabbit.—The glans penis, prepuce, vulva, and perineal pouches were examined. The genital organs themselves were in all cases

very clean. The perineal pouches, situated on either side of the genitals, contain a large collection of a yellowish, soft, granular, oily material having a peculiar penetrating odor—the rabbit odor. This material microscopically is seen to be made up of numerous oily droplets, of squamous epithelial cells, which are more or less swollen, and of many kinds of bacteria. Notwithstanding the large amount of oily material in the perineal pouches, in the ten rabbits examined not a single acid-resisting bacillus was found. This fact is of interest in view of the common belief that fat is necessary for the staining reaction of the smegma bacillus. The fat in smegma, however, generally exists in a saponified form. The mammae of five rabbits were also examined but with negative results.

Cat.—In the cat, as in the rabbit, the genitals are not exposed, and are very clean, and it is with difficulty that a cover-glass smear can be made. A few bacteria which stain blue are all that can be seen. Two male cats were examined with negative results.

White Rat.—On the prepuce when retracted, and at times on the external surface near the meatus, there may be seen small yellowish semisolid lumps about the size of a common pin head. Microscopically this material contains swarms of very short, thick, acid-resisting bacilli. These may be seen in groups of one hundred or more, or scattered singly through the field. A simple examination of these acid-resisting bacilli shows that several varieties or species are present. Thus some resemble diplococci, others have a drumstick form, while others have a central, clostridium-like enlargement. Seven males were thus examined with positive results in five, negative in two. The female shows no special material or smegma, and it is only with the greatest persistence that any material is obtained. The vagina at times contains a mucous substance. Three females examined showed positive results in one, negative in two.

The feces of the rabbit, guinea-pig, and white rat was examined with negative results.

Many are accustomed when speaking of the smegma bacillus, to think of it as a typical organism like the tubercle bacillus. This is not correct inasmuch as we have to deal with a group of bacilli which differ markedly among themselves. As in man, so in the lower ani-

mals, we find in the smegma various forms of microorganisms which in spite of comparatively long exposure to acid solutions retain their color, whereas the other bacteria with which they are associated decolorize readily. The extreme variation in the form of these acid-resisting bacilli clearly indicates a difference in species.

General Morphology.—The smegma of the lower animals very often contains slender, clean-cut, slightly bent rods which frequently show a distinctly beaded arrangement, and in this respect are not to be distinguished from the tubercle bacillus. On the other hand, smegma bacilli are met that are appreciably shorter or longer than the tubercle bacillus. Moreover, irregular forms can be observed, the bacilli instead of being straight may be bent in the middle; this bend may be scarcely perceptible (comma bacillus), or may be almost a right angle. At times the stain is taken up unevenly and as a result the bacilli show irregular borders.

The occurrence of acid-resisting diplococci or diplo-bacilli and of drumstick forms has been already referred to. Oval, yeast-like organisms, capsulated bacteria, short threads of from four to six cells, and spores are met which retain the stain the same as the ordinary smegma bacillus. The organisms found on parts of the body other than the genital organs, as on the teats of the cow, usually vary in form as much as those in smegma.

Decolorization.—In the resistance which the smegma bacillus and other forms referred to offer to decolorization by acids, these organisms resemble the tubercle and leprosy bacilli. At one time the tubercle bacillus was supposed to have a specially impermeable cell wall, but this view has gradually given way to the belief that the peculiar reaction is due more to the composition of the cell contents than to the cell wall proper. That the resistance of the smegma bacillus to decolorization is not inconsiderable is seen in the fact that specimens of smegma can be left in 25 per cent nitric acid solution for ten minutes without the least decolorizing effect. The same is true of the bacilli found in the scrapings from the cow. Invariably the specimens will resist exposure for three to five minutes to this acid solution, or to the same strength of sulphuric and of acetic acids. An exposure of ten seconds to absolute alcohol decolorizes the bacilli

in the majority of cases, but at times a much longer exposure has practically little decolorizing effect.

As has been stated, some of the acid-resisting bacilli which are met among the lower animals resemble in form the tubercle bacillus. In the staining properties and especially in the resistance to decolorizing solutions this resemblance is rendered more striking. Nevertheless it would be a gross error to conclude that the tubercle bacillus was actually present. It is quite possible that the real tubercle bacillus may have been present, but its recognition by the only crucial tests—cultures and growth in the animal body—was not resorted to, for the reason that the immediate object in view was to demonstrate the presence of acid-resisting bacilli on the lower animals.

The presence of acid-resisting bacilli in milk or in butter may mislead and doubtless has misled those who depend entirely upon the recognition of the tubercle bacillus by its morphological and staining properties. Reports of the presence of tubercle bacilli in milk and in butter based on such observations are by no means few. The mere detection of such bacilli in milk or butter is now recognized as giving no support to the conclusion that they are tubercle bacilli. In order to identify such acid-resisting bacilli it is necessary to resort to cultures and animal experiments, but even here a certain amount of caution must be observed, not to mistake nodules produced by injection of butter and pseudo-tubercle bacilli for genuine tubercles.

CONCLUSIONS.

The results obtained in this study may be briefly summarized:

1. Acid-resisting bacilli are found in many of the lower animals, more especially the horse, cow, dog, guinea-pig and white rat. In the case of the rabbit and cat no such organisms were detected.
2. Many of these acid-resisting bacilli resemble the tubercle bacillus and the smegma bacillus of man.
3. The acid-resisting organisms are undoubtedly of different species and there is good reason to believe that the term smegma bacillus denotes not a definite species but rather a group of bacilli having common staining properties.

A COMPARATIVE STUDY OF THE BIOLOGICAL CHARACTERS AND PATHOGENESIS OF BACILLUS X (STERNBERG), BACILLUS ICTEROIDES (SANARELLI), AND THE HOG-CHOLERA BACILLUS (SALMON AND SMITH).*

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PLATE XIX.

CONTENTS.

	PAGE
INTRODUCTION	216
I. BACILLUS X.....	216
1. Characters	217
2. Pathogenesis	219
3. Lesions	226
II. BACILLUS ICTEROIDES AND THE HOG-CHOLERA BACILLUS	229
1. Comparison of their characters.....	230
2. Comparative pathogenesis	236
(a). Mice.....	236
(b). Guinea-pigs and rabbits ...	237
(c). Dogs.....	242
(d). Pigeons.....	248
(e). Swine.....	249
3. Reciprocal immunization	259
(a). Immunization of guinea-pigs from the hog-cholera bacillus with sterilized cultures of <i>B. icteroides</i>	260
(b). Immunization of guinea-pigs from <i>B. icteroides</i> with sterilized cultures of the hog-cholera bacillus	260
(c). Immunization of rabbits from the hog-cholera bacillus with living cultures of <i>B. icteroides</i>	263
4. Reciprocal agglutinative reactions.....	264
(a). Sera of animals immunized from <i>B. icteroides</i> and the hog-cholera bacillus.....	264
(b). Blood-serum of yellow fever patients	265
(c). Blood-serum of hog-cholera.....	267
III. CONCLUSIONS.....	269
DESCRIPTION OF PLATE XIX.....	270

* Received for publication February 25, 1900.

The observations which here follow were begun under the direction of Surgeon-General Sternberg for the purpose of critically comparing both the cultural characters and pathogenic action of his *Bacillus X*, derived from yellow-fever cadavers, with those of *Bacillus icteroides* (Sanarelli), which had been recently announced as the specific agent of yellow fever.¹

During this investigation our attention was attracted to the remarkable cultural resemblances of *Bacillus icteroides* and the hog-cholera bacillus, which led us to take up a new line of comparative experiments with these bacilli.

In a preliminary note, published in the *Medical News*, April 29, 1899, we have briefly called attention to some of the cultural resemblances of these bacilli and the similarity of the lesions produced in the guinea-pig, rabbit and dog by *Bacillus icteroides* to those found in these animals after inoculation with the hog-cholera bacillus. We also noted the marked agglutinative reaction which the serum of an animal immunized with *Bacillus icteroides* exerted toward the hog-cholera bacillus; and we recorded the fact that the former bacillus, when fed to the domestic pig, would bring about an acute fatal infection in which the principal lesion was found in the large intestine.

As the result of the comparative study which we had made at that time of *Bacillus X* (Sternberg) and *Bacillus icteroides* (Sanarelli), we expressed the opinion that while the former belonged to the colon group, the latter should be considered as a variety of the hog-cholera bacillus, and hence as a secondary invader in yellow fever.

In this report we propose to give more at length the observations upon which these conclusions are based.

I.

BACILLUS X.

Since the cultural characters of *Bacillus X* are in striking contrast to those of *Bacillus icteroides* and the hog-cholera bacillus, we have

¹Sanarelli. A lecture on yellow fever, with a description of the *Bacillus icteroides*. *British Medical Journal*, 1897, iii, p. 7.

concluded to give this bacillus separate consideration. The difference in its pathogenic action, as manifested toward the smaller animals, is an additional reason for adopting this course.

The culture of this bacillus which we have used in our comparative experiments was received by Dr. Sternberg from Dr. E. H. Wilson, of the Hoagland Laboratory, Brooklyn, New York, where it had been kept as a stock culture for about four years. The original culture had been isolated by Sternberg,² during the summer of 1889, in the city of Havana, from yellow-fever cadavers—Cases Nos. 18 and 28. It had not been isolated by direct culture from the cadaver, but from the serous effusion beneath the skin and from the livers of the guinea-pigs which had died from the subcutaneous inoculation of a small quantity of the blood and crushed liver parenchyma of cases of yellow fever autopsied soon after death. When placed in our hands, Bacillus X had been cultivated on artificial media for about seven years.

CHARACTERS OF BACILLUS X.—*Morphology.*—As originally described by Sternberg, Bacillus X “resembles the colon bacillus in form, but is somewhat larger, from 2 to 4 μ in length by 0.8 to 1 μ in diameter; sometimes occurs in pairs; may grow out into short filaments, not common.” It is stated that “in recent gelatine cultures it is often so short an oval in form that it might be mistaken for a micrococcus.” The morphological characters of Bacillus X as observed in our cultures agree with this description. The bacillus is decolorized by Gram’s method.

Motility.—Bacillus X exhibited active movements in cultures made directly from the yellow-fever cadavers. This motility was not constant and was not observed in cultures which were brought from Cuba to the United States. Sternberg states: “These movements were usually not observed in all the bacilli in a field under observation, but one and another would start from a quiescent condition on an active and erratic course, sometimes spinning actively upon its axis, and again shooting across the field as if propelled by a flagellum.” It was noted that cultures passed through the guinea-pig were more apt to be motile.

We have not observed motility in this bacillus. For the purpose of reviving its active movements we have repeatedly passed it through guinea-pigs, but without success as regards the restoration of motility. When stained by suitable methods, however, such as Pittfield’s, flagellate forms, few in number, may be seen. The number of flagella varies from 1 to 6.

² Report on the Etiology and Prevention of Yellow Fever. Washington, 1890, pp. 187–200.

Bouillon.—*Bacillus X* grows freely in this medium, which becomes heavily clouded without the formation of a surface pellicle. After 48 to 72 hours a small, somewhat dense deposit is found at the bottom of the tube. The bacillus grows rapidly in bouillon to which glucose, lactose or saccharose has been added, with the appearance of numerous small gas bubbles at the surface of the liquid.

A disagreeable odor, resembling that of the colon bacillus, is given off by all cultures of *Bacillus X*.

Gelatine.—In stick-cultures, the growth resembles that of the colon bacillus. The surface growth may consist of a soft milk-white layer, or may be quite thin and dry, with very irregular outline. The tendency of the growth is to overspread the surface of the medium. Abundant development occurs all along the line of puncture and presents a yellowish color by transmitted light. Delicate tufted outgrowths frequently appear along the line of puncture.

In gelatine plates, the deep colonies, after 24 hours at 20° C., may readily be detected with the eye as minute white points. Under the low power they appear as round, finely granular masses, of a pale yellow color. Older deep colonies become opaque, change their color to a dark brown and often show a tendency to become lobulated. Surface colonies after 24 hours are of a pale or faintly yellow color, finely granular throughout, with irregular margin. Some of these colonies may show a delicate veining. After 48 hours, surface colonies are 2 to 3 mm. in diameter, and of a pearl-gray color by reflected light. Under the microscope they show dense granular, brown centres, surrounded by a lighter colored portion that is distinctly veined. The central part of the colony shades off gradually into a pale, spreading, irregular margin. In the smallest surface colonies, the peripheral portion is broader, paler and slightly indented, while the central part has a yellowish tinge and is delicately veined, giving a striking resemblance to young colonies of the colon bacillus. Old surface colonies, by reflected light, are glistening, raised, of a milk-white color and irregular outline. Under the low power they show a dark-brown, granular centre surrounded by a colorless, irregular margin.

Acid Potato-Gelatine.—The bacillus grows freely as a dense, grayish-white, shining layer, slightly raised above the surface.

Acid Potato-Gelatine Containing 1 per cent KI.—Develops readily on this medium.

Agar-Agar.—In stroke cultures the growth appears as a moist, soft, grayish-white, raised layer which, while spreading to some extent on either side of the line of inoculation, does not show a tendency to over-

spread the medium. The margins are frequently thin and notched. This growth on agar closely resembles that of the colon bacillus.

On agar plates, the deep colonies are round, oval or whetstone-shaped, of a brownish color, finely granular and sometimes show a tendency to lobulation.

At thermostat temperature, after 24 hours, the surface colonies are from 2 to 4 mm. in diameter and by reflected light of a grayish, or milk-white color. Under the low power they are finely granular, of a brownish-yellow color and often show a distinct veining; the margin is irregular in outline. These spreading colonies resemble those of the colon or typhoid bacillus.

Potato.—Grows rapidly on potato; at first, as a thin, moist, slightly brownish or yellowish-brown layer, with tendency to overspread the surface of the medium; later, the growth while remaining quite moist, becomes more raised and presents a dirty-grayish, or grayish-yellow color; no gas bubbles are to be seen at any time.

Milk.—Grows well in this medium, which is generally coagulated at the end of 2 to 6 days at thermostat temperatures. Litmus milk is decolorized and slightly reddened at the end of 24 hours. At the expiration of 48 hours coagulation has begun, and is generally complete after 3 to 6 days. We have observed that cultures which have recently been passed through animals have, as a rule, brought about the coagulation of milk more slowly.

Fermentation Tube; Glucose Bouillon 1 per cent.—Grows rapidly in glucose bouillon, producing prompt and marked fermentation. At the end of 24 hours, the amount of gas present in the closed branch of the tube varies from 4.5 to 6 cm. There is but little increase in the quantity at the expiration of 48 and 72 hours. Reaction strongly acid.

Lactose, 1 per cent.—This medium is also promptly fermented. At the end of 24 hours the quantity of gas equals 4.5 to 5 cm. This is only slightly increased after 48 to 72 hours. Reaction acid.

Saccharose, 1 per cent.—This medium is at first slowly fermented. The quantity of gas at the end of 24 hours does not exceed 0.5 cm. During the second day the fermentation is more rapid, amounting to about 3 cm. at the end of 48 hours. This may be slightly increased at the end of 72 hours. Reaction acid.

Indol Reaction.—In Dunham's solution *Bacillus X* produces indol. This reaction is not seen after the addition of sulphuric acid alone, but requires the addition of a nitrite in order to bring about the change in color, which is less intense than is usually seen with the colon bacillus.

PATHOGENESIS.—Our experiments have been confined to guinea-pigs, rabbits and dogs.

Guinea-pigs. (a) *Subcutaneous Inoculation.*—Sternberg found that this bacillus was pathogenic for guinea-pigs when injected into the cavity of the abdomen in doses of 2 to 3 cc. of a culture in agua coco, but that the subcutaneous injection of 0.5 to 1 cc. gave negative results in 11 out of 13 guinea-pigs inoculated. Two died within 24 hours.

The fact that the animals were not kept under observation longer than one week will probably account for the failure to record a larger number of deaths from the subcutaneous injection of *Bacillus X*, since we have found that this bacillus when injected subcutaneously in quantities varying from 1 cc. to 5 cc. of a 24-hour bouillon culture, is almost always pathogenic for guinea-pigs.

The animals for a few days following the injection may appear quiet and refuse food, which is frequently the case; or they may exhibit no symptom of illness. Although the guinea-pigs do not appear to be sick, carefully recorded observations show that there is a steady loss of weight. The period within which the animals die, however, is a very uncertain one, varying from 1 to 7 weeks. Exceptionally, a guinea-pig has survived the subcutaneous injection of 1 to 2 cc. of *Bacillus X*.

We give brief protocols of some of our experiments:

Exp. I.—July 16, 1897. Guinea-pig No. 315; weight 300 grammes. Injected subcutaneously with 2 cc. of a 24-hour bouillon culture of *Bacillus X* (original). Death after 7 days. Site of inoculation marked by necrosis of skin, $2\frac{1}{2} \times 1\frac{1}{2}$ inches. Subcutaneous tissues moist and injected. Inguinal lymph glands enlarged and pale. Liver congested, markings indistinct. Spleen small, pale. Kidneys swollen and injected. Adrenals enlarged and hyperæmic. Mucous membrane of small intestine congested. Cultures from blood, liver and urine positive, a few colonies of *Bacillus X* being obtained from each of these sources. Cultures from bile, spleen and kidney negative.

Exp. IV.—August 9, 1897. Guinea-pig No. 348; weight 377 grammes. Injected subcutaneously with 4 cc. of a 24-hour bouillon culture from liver of dog No. 347. Death after 37 days. Animal emaciated. Axillary and inguinal glands enlarged. Parietal peritoneum injected. Liver congested. Spleen normal. Kidneys and adrenals enlarged and hyperæmic. Lungs deeply congested. Stomach contains a small quantity of mucoid, blood-stained material; mucous membrane generally injected. Mucous membrane of small intestine deeply congested throughout. Large intestine and appendix normal. Cultures from blood, spleen, liver, kidney, bile and urine all negative.

After repeated passage through the peritoneal cavity of guinea-pigs, the virulence of the original culture of this bacillus was perceptibly in-

creased, so that death most often occurred within 8 to 9 days after the subcutaneous injection of 1 cc. of a 24-hour bouillon culture. In order, however, to bring about a fatal result in less time it was necessary to inject a larger quantity of this more virulent culture.

When death has occurred within 3 to 7 days, *Bacillus X* has been recovered from the site of inoculation in large numbers, and in fewer numbers from the blood and internal organs as well as from the urine and bile. From guinea-pigs that have survived longer than 8 days we have failed to recover this bacillus in culture, except in one instance where death occurred on the 26th day after injection. In this case cultures from the liver and bile only were positive.

(b) *Intraperitoneal Inoculation*.—We have found *Bacillus X* to possess more marked pathogenic action for guinea-pigs when injected into the peritoneal cavity. While the original Hoagland culture, injected by this method in doses of 1 to 5 cc., did not produce death until after considerable intervals, cultures recently obtained from animals sufficed to bring about a fatal result at shorter periods. As a rule, death occurs within 24 hours in medium-sized guinea-pigs injected intraperitoneally with 1 cc. of the more virulent cultures. The animal is profoundly affected within a few hours after the injection, remains quiet, and dies at intervals varying from 10 to 24 hours. At autopsy there are found serofibrinous peritonitis and marked congestion of the abdominal viscera.

Exceptionally, this quantity of virulent culture has failed to produce death in guinea-pigs weighing 500 grammes until after a period of 3 or more weeks.

From animals that have resisted the peritoneal injection longer than 8 days, we have generally failed to obtain the bacillus in culture. From those dying within shorter intervals, and especially within 24 hours, the organism has been recovered in pure culture from the blood, liver, spleen, kidney, bile, urine and abdominal cavity. The number of colonies has been more abundant from the abdominal cavity than from other sources; hence, although the blood and internal organs may be invaded by *Bacillus X* in animals that are overwhelmed with large doses, our experience has shown that where life is more prolonged, the number of bacilli undergoes rapid reduction in the abdominal cavity as well as in the blood and organs.

Our observations have led us to believe, therefore, that *Bacillus X* does not multiply to any considerable extent in the bodies of inoculated guinea-pigs, but that death is brought about sooner or later by the action of its toxic products. To this we shall again recur.

Rabbits. Sternberg observed that *Bacillus X*, when injected into the

cavity of the abdomen in doses of 1 to 10 cc., was quite pathogenic for rabbits, the animal frequently dying within a few hours. This applied to

TABLE I.
RABBITS INOCULATED WITH BACILLUS X.

No.	Weight in grammes.	Method.	Quantity.	Result.	Lesions.	Cultures.
1	642	Subcutaneous.	2 cc. 24-hour culture.	Death after 3 days.	Abcess at site of inoculation; lymph glands enlarged; liver engorged; spleen slightly enlarged; kidneys congested; adrenals normal; mucosa of upper part of small intestine congested.	Wound: numerous colonies. Spleen and urine: few colonies. Blood, liver, bile, and kidney negative.
2	1040	Subcutaneous.	5 cc. 24-hour culture.	Death after 3 days.	Same lesions as noted above.	Cultures from wound and urine positive; other sources negative.
3	600	Abdominal cavity.	5 cc. 24-hour culture.	Death after 25 hours.	General serofibrinous peritonitis; punctate hemorrhages over small and large intestines; liver engorged; spleen small; kidneys congested; thymus gland much enlarged.	Cultures positive.
4	525	Abdominal cavity.	2 cc. 24-hour culture.	Death after 11 days.	Animal emaciated; no peritonitis; liver congested; spleen small; intestine normal.	Cultures negative.
5	527	Intravenous.	3 cc. 24-hour culture.	Death after 7 days.	Rabbit emaciated; liver and kidneys congested; spleen small; increased fluid contents in small intestine, its mucosa slightly injected.	Cultures negative.

cultures recently isolated from yellow-fever cadavers. Of 27 animals inoculated, only 3 recovered. Subcutaneous and intravenous inoculations with small doses (2 to 4 minims) of a fluid culture were negative.

One cc. of an *agua coco* culture, subcutaneously, was fatal at the end of 30 hours. He concludes that the negative results obtained by subcutaneous and intravenous inoculations show that *Bacillus X* does not induce septicæmia in the rabbit, and the fatal result of intraperitoneal injections is due rather to its toxic products than to invasion of the blood. Direct examination of the blood of rabbits which had succumbed within a few hours, showed very small numbers of the bacilli. No enlargement of the spleen was found.

We have found this bacillus pathogenic for rabbits in quantities of 1 to 5 cc., whether injected subcutaneously, into the cavity of the abdomen, or intravenously.

The results of the inoculation of rabbits with this bacillus are shown in Table I.

The intervals within which rabbits have died after the subcutaneous inoculation of *Bacillus X* have varied considerably. In some cases the animals have survived from 16 to 43 days. In a few experiments the only effect has been the local formation of pus at the site of inoculation. The result of cultures would indicate that this bacillus, when inoculated subcutaneously in rabbits, shows little tendency to invade the blood. While large quantities (5 cc.) injected into the abdominal cavity generally cause a fatal peritonitis and death within 24 hours, smaller quantities (1 to 2 cc.) do not bring about a fatal result until after a considerable interval (1 to 3 weeks). Cultures from animals that have survived longer than one week have generally proved sterile. In a few young rabbits inoculated intravenously, death has occurred within 36 to 43 hours with positive cultures from blood and organs; while in older animals a fatal result has generally been brought about after the lapse of from 12 to 20 days with negative cultures from all sources. In all chronic cases emaciation has been a prominent feature.

The results of the inoculation of rabbits with *Bacillus X* indicate that, as with the guinea-pig, this organism does not multiply to any extent in the blood, but that death is brought about by toxæmia. This conclusion is supported by the results obtained by the inoculation of rabbits and guinea-pigs with filtrates from cultures of *Bacillus X* grown for a period of 40 days at thermostat temperature. The results are briefly recorded in Table II.

Aside from a small quantity of blood-stained serum in the abdominal cavity, no post-mortem lesion was noted. These experiments serve to show that death may be rapidly produced in guinea-pigs and rabbits by the toxic substances contained in old filtered cultures of *Bacillus X*.

Dogs.—Our experiments with these animals have been confined to the

intravenous injection of *Bacillus X*, and were made for the purpose of comparing the results with those obtained by the intravenous inoculation of *Bacillus icteroides* in dogs. We have inoculated by this method 11 dogs, of which number 6 have died and 5 recovered. We submit protocols of a few of these experiments.

TABLE II.

GUINEA-PIGS AND RABBITS INJECTED WITH FILTRATES FROM CULTURES OF
BACILLUS X.

Animal.	Weight. in grammes.	Quantity.	Method.	Result.	Cultures.
Guinea-pig.	276	5 cc.	Abdominal cavity.	Death after 7 hours.	Sterile.
Guinea-pig.	263	5 cc.	Abdominal cavity.	Death after 12 hours.	Sterile.
Guinea-pig.	638	5 cc.	Subcutaneous.	Death after 15 days.	Sterile.
Guinea-pig.	642	5 cc.	Subcutaneous.	Recovery.	
Rabbit.	485	10 cc.	Abdominal cavity.	Death after 10 hours.	Sterile.
Rabbit.	580	15 cc.	Abdominal cavity.	Death after 35 hours.	Sterile.
Rabbit.	840	20 cc.	Abdominal cavity.	Recovery.	

Exp. I.—July 16, 1897, 11.30 A. M. Dog No. 318; weight 13 lbs. Inoculated into ear vein with 10 cc. of a 24-hour 1 per cent glucose bouillon culture of *Bacillus X* (original). When returned to its cage, the dog appeared dejected and sick. 1 P. M., animal drowsy; vomited freely at this hour. 2.30 P. M., again vomited a frothy mucous fluid. July 17, 9 A. M., dog quiet, rectal temperature 101° F. During the day became brighter and took its food. Recovered.

Exp. II.—July 23, 1897, 1.20 P. M. Young dog No. 327; weight 13 lbs. Injected into the ear-vein with 5 cc. of a 24-hour glucose bouillon culture of *Bacillus X* from blood of rabbit No. 314. Lively, active dog. At 1.35 P. M. animal appears quite sick, is lying on its side; does not respond to the voice. 2.05 P. M., vomits freely partly digested food, followed by fluid action from the bowels with tenesmus. 2.23 P. M., small, brown, watery stool; this repeated after a short interval with marked tenesmus. 2.51 P. M. and 3.05 P. M., vomits with much effort a small quantity of grayish frothy fluid mixed with mucus. 3.30 P. M., again vomits. 4 P. M., dog lies on its side with extremities extended; temperature at this hour 104.1° F. Temperature previous to injection 101° F. Death 6 P. M., 28½ hours after the inoculation.

Autopsy.—Thorax: Thymus gland large, dark red in color, with few

small hæmorrhagic areas on surface. Mediastinal glands swollen, of a dark-red color. Both layers of pericardium injected. Small hæmorrhagic area over right auricle, which is distended with blood; left auricle empty. Right ventricle distended; left ventricle contracted; numerous hæmorrhagic areas beneath endocardium in this ventricle; valves normal. Myocardium pale red. Both lungs congested; lower lobe of right lung œdematous. On cut section, reddish serous fluid exudes freely. Small hæmorrhagic areas under the visceral pleura over the lower lobe right lung.

Abdomen: Omentum injected. Numerous small hæmorrhages under serous coat of small intestine. Liver mottled, pale and red; light areas most extensive; outlines of lobules distinct; central veins appear injected; peripheries of lobules of pale, yellowish color. Spleen enlarged, dark red, firm. Adrenals, small, pale. Gall-bladder moderately full of dark brownish bile. Kidneys enlarged; cortex swollen, pale; pyramids injected. Stomach contains small quantity of bile-stained fluid; mucous membrane over greater curvature dark red in color; injection uniform. Small intestine, duodenum and upper part of jejunum contain considerable quantity of soft, black, tarry material; mucous membrane pale throughout. Peyer's patches not swollen. Beginning at the ileocæcal valve and extending to anus the rugæ of mucous membrane of large intestine are the seat of marked hæmorrhages which extend into the submucosa. Small quantity of fluid blood in the large intestine. Bladder contracted; contains about 4 cc. of albuminous urine.

Cultures from the blood, liver and spleen give numerous colonies of *Bacillus X*; urine and bile negative.

Exp. III.—August 6, 1897. Dog, weight 10 lbs. Injected at 2.45 P. M. with 13 cc. of a 72-hour 2 per cent lactose bouillon culture of *Bacillus X* from rabbit No. 338. 3.05 P. M., animal restless; vomits partially digested food with much retching; 3.15 again vomits, followed by watery stool mixed with mucus; considerable tenesmus; 4 P. M., rectal temperature 96.4° F. Before the injection, temperature 101° F. Found dead the following morning at 8 o'clock, less than 18 hours after inoculation.

Autopsy.—Thorax: Lungs slightly injected. Several small hæmorrhagic areas beneath pleural surface of upper lobe of right lung. Subendocardial hæmorrhages in the left ventricle.

Abdomen: Liver of a pale, grayish color; cut surface dry, markings indistinct. Spleen slightly enlarged, dark red, soft. Kidneys swollen; on cut section injected throughout. Stomach contains about 200 cc. of fluid blood, mucous membrane dark red throughout. Much fluid

blood in both small and large intestine. Mucous membrane of small intestine swollen, dark red in color. Less injection in the large intestine. Bladder contracted, empty.

Cultures from the blood, liver, spleen and urine positive; bile and kidney negative.

In another experiment with a young dog, weight $7\frac{1}{2}$ lbs., injected into the ear-vein with 3 cc. of a 24-hour bouillon culture from dog 347, death occurred after 11 hours, with intense hæmorrhagic gastro-enteritis.

Four of our dogs have died from a single injection of *Bacillus X*, one after a second injection and one after repeated injections.

The clinical picture has been the same in all of the dogs injected, namely, vomiting, increased action of the bowels with rectal tenesmus and marked prostration. No after effects have been observed in dogs that have recovered, the animals appearing to regain their appetite and strength within two or three days.

LESIONS. (a) *Macroscopic*.—We have already sufficiently indicated, in the foregoing protocols, the gross lesions to be seen at autopsy in animals inoculated with *Bacillus X*. Briefly recapitulating these, we may say that in guinea-pigs the lesions are injection of the subcutaneous vessels at the site of inoculation, or sometimes local abscess or sloughing of the skin; enlargement of the inguinal and axillary lymph-glands; injection of the parietal peritoneum and of the mucous membrane of the stomach and small intestine; congestion of the lungs, and, as a rule, engorgement of the liver and kidneys. Hyperæmic swelling of the adrenals has always been present. The spleen is generally small, pale and firm, although occasionally slightly enlarged and dark in color.

In the rabbit, aside from local abscess-formation at the site of inoculation and swelling of the lymph-glands, the most prominent and constant post-mortem finding has been congestion and swelling of the mucous membrane of the small intestine, accompanied by increase in the fluid contents of the small bowel, and sometimes with areas of hæmorrhage beneath its serous coat.

In animals dying within a short interval following the inoculation, the liver and kidneys have been congested, while the spleen has been small or only slightly enlarged. Enlargement of the thymus gland has occasionally been noted.

In both guinea-pigs and rabbits, intraperitoneal inoculation has usually been followed by a serofibrinous peritonitis.

In dogs the hæmorrhagic lesions have been much more pronounced than in the rabbit or guinea-pig. These have consisted of numerous small hæmorrhages beneath the endocardium, under the visceral layer

of the pleura and scattered over the surface of the small intestine. In addition, intense engorgement of the mucous membrane of the stomach and small intestine, with frank hæmorrhage into the lumen of these viscera has occurred in several cases. In one instance this congestion with hæmorrhage into the submucosa was confined almost entirely to the large intestine. Hæmorrhagic areas involving the vesical mucous membrane have also been noted in dogs. The liver has been pale, or mottled yellow and red, while the kidneys have been swollen and injected. The spleen has been slightly enlarged and soft, or small and firm; the adrenals pale and small.

(b) *Microscopic*.—For the purpose of microscopic examination, tissues have been hardened in absolute alcohol, 5 per cent formalin, Orth's fluid and Flemming's osmic solution. Also fresh frozen sections of the several organs have been examined in normal salt solution for the purpose of detecting any fatty change.

As the result of careful microscopic examination, there are few lesions to record in the guinea-pig and rabbit. No changes from the normal have been found in the spleen. Sections of the lymph-glands and of the adrenals in guinea-pigs show marked dilatation of the blood-vessels of both cortex and medullary portion. No finer lesions have been discovered in these structures. The same engorgement of the lymph-nodes has been observed in the rabbit, whereas the adrenals have shown no change. As in other acute intoxications, moderate granular and fatty degenerations have been seen in the hepatic cells, but in this organ the most prominent feature has consisted in engorgement of the intralobular capillaries. In some instances this dilatation of the capillaries has been so great as to lead to compression and narrowing of intervening rows of cells. Circumscribed hæmorrhages in the liver have also been observed. Small foci of coagulative necrosis were found in the liver of one rabbit that died on the 20th day after intravenous injection. These areas of necrotic cells were small, few in number, and situated within the lobules. No changes in the kidney have been observed save dilatation of the blood-vessels and cloudy swelling of the secreting epithelium in acute cases.

In dogs that have died within a short time after intravenous injection (11 to 13 hours) no lesions were observed in sections of the liver other than granular degeneration of the cells, but in animals that have died after longer intervals (28½ hours to 3½ days), in addition to some fatty degeneration there is present extensive coagulative necrosis of the hepatic cells. The distribution of the necrotic areas, as in other acute infections, is quite variable. While some of these are situated about the

central vein, involving part or the entire circumference of the latter, other foci are located within the lobules. At times an entire lobule is included in the necrosis; again, parts of adjacent lobules. Within these areas the liver-cells stain brightly with eosin and appear as swollen, quite refractive bodies, some of them containing minute fat-drops. No nucleus can be seen in many of these cells, or it may appear as a pale shadow, or much contracted and staining deeply with hæmatoxylin. In some of the cells the nucleus has undergone fragmentation. Within the capillaries of the affected area, a few small cells with round, normally staining nuclei are to be seen. The number of leucocytes within the capillaries does not appear to be increased, and there is an entire absence of any invasion of the necrotic foci by these cells. Necroses of single cells were also observed in sections of the dog's liver. The changes in the kidney were limited to parenchymatous degeneration of the secreting epithelium. The gross lesion in the intestine of the dog consisted of swelling and intense injection of the mucous membrane with hæmorrhages. The microscopical changes were confined to a rather free desquamation of the epithelium covering the villi and that lining Lieberkühn's follicles, and marked dilatation of the blood-vessels of the mucosa and submucosa. Small hæmorrhages into the superficial layer of the submucosa were also observed.

Referring to the gross lesions found in guinea-pigs and rabbits that have died after inoculation with *Bacillus X*, it will be seen that these agree with the appearances found by Escherich,³ Emmerich,⁴ Blachstein,⁵ and other observers who have inoculated these animals with bacilli belonging to the colon group. The varying length of time during which the animals have lived after receiving the inoculation and the negative result of cultures in those that have survived for considerable periods, also agree in the main with our results.

We have not been able to find in the literature any reference to the intravenous injection of dogs with a member of the colon group. Emmerich, however, observed in dogs that were injected subcutaneously with considerable quantities of his *Bacillus Neapolitanus*, repeated vomiting, profuse diarrhœa and prostration. Death occurred in one of three thus inoculated, with ulceration of the mucous membrane of the small intestine and enlargement of the solitary follicles.

For the purpose of comparative experiment, we have inoculated one

³ Arbeiten aus dem pathologischen Institut zu München, p. 68. Stuttgart, 1886.

⁴ Untersuchungen über die Pilze der Cholera asiatica. *Arch. f. Hygiene*, 1885, iii, p. 313.

⁵ *Bulletin of the Johns Hopkins Hospital*, 1891, ii, p. 96.

dog intravenously with 5 cc. of a 4-hour plain bouillon culture of *Bacillus coli communis*, recently isolated from the abdominal cavity of a patient who had died from general peritonitis. The clinical symptoms observed in this dog corresponded to those seen in dogs injected with *Bacillus X*, namely, repeated vomiting, increased action of the bowels with rectal tenesmus, prostration and rise of temperature. This animal, although apparently quite sick for two days, made a good recovery.

That *Bacillus X*, after cultivation on artificial media for about seven years was still virulent for the smaller animals, appeared to us as hardly to be expected in a member of the colon group. It seems, however, that this retention of virulence has been shown by certain colon bacteria which have been kept in cultivation for even longer periods. Thus Novy⁶ found that 1 cc. of a 24-hour bouillon culture of Emmerich's bacillus which had been cultivated on artificial media for a period of 10 years would bring about death within 18 hours when injected into the abdominal cavity of the guinea-pig.

Recalling the important biological characters of *Bacillus X*, viz., the slight motility observed in recently isolated cultures; the appearance of colonies in gelatine; the coagulation of milk; the fermentation of glucose, lactose and saccharose; the production of indol and its decolorization by Gram's method, we think that these, together with the results obtained from animal experimentation, are sufficiently distinctive to warrant us in placing this bacillus in the colon group.

II.

BACILLUS ICTEROIDES AND THE HOG-CHOLERA BACILLUS.

The culture of *Bacillus icteroides* with which we have made the majority of our experimental observations, was obtained by Dr. Sternberg from the laboratory of Professor Roux, in Paris. When received by us, it bore the label of the Laboratory of Hygiene of the University of Montevideo. This culture we have transplanted from time to time on agar-agar, and have labeled it "*Bacillus icteroides*, original."

We have also received, through the courtesy of Dr. A. Agramonte, Acting Assistant Surgeon, U. S. Army, two cultures of *Bacillus icteroides*, one of which was isolated from the cadaver of Private Patrick Smith, 8th U. S. Infantry, who died in Havana, and concerning the

⁶ The Etiology of Yellow Fever. *Medical News*, 1898, lxxiii, p. 330.

diagnosis of whose case there was much uncertainty on the part of his medical attendants; the other, from a yellow-fever cadaver at Santiago, Cuba. These latter cultures we have designated "Bacillus icteroides, Havana," and "Bacillus icteroides, Santiago," respectively.

The cultures of the hog-cholera bacillus with which we have made comparative observations, were obtained from the Bureau of Animal Industry, Washington, D. C., through the courtesy of Dr. E. A. de Schweinitz, and from the Pathological Laboratory of the Johns Hopkins University, through the kindness of Dr. Harvey Cushing. These cultures we have designated "Hog-cholera No. 1," and "Hog-cholera No. 2," respectively.

Comparison of the Characters of Bacillus Icteroides and the Hog-cholera Bacillus.

Each of these organisms is a facultative anaërobic bacillus which decolorizes by Gram's method, and does not liquefy gelatine.

Morphology.—According to Sanarelli,⁷ *B. icteroides* appears as short rods, with rounded extremities, generally united in pairs, from 2 to 4 μ in length and, as a rule, twice as long as broad. Salmon and Smith⁸ describe the hog-cholera bacillus as consisting of short rods, round at the ends, chiefly in pairs, measuring from 1.2 to 1.8 μ in length by 0.6 μ in breadth. The size of each of these bacilli, however, varies much according to the particular medium on which it is grown. In cultures on potato, much longer and thicker forms are shown by both.

Motility.—Both of these bacilli show very active movements.

Sanarelli gives the number of flagella for *B. icteroides* as 4 to 8. Moore,⁹ who carefully studied the flagella of the hog-cholera bacillus, from a large number of counts places the average number of flagella as 3.3; the majority of the flagellate forms showed 3 to 6 flagella; some as many as 8 to 11 flagella.

Bouillon.—We have noticed no difference in the growths of *B. icteroides* and of the hog-cholera bacillus in flesh-peptone bouillon. This medium is only moderately clouded by both of these bacilli. No surface pellicle, as a rule, is to be seen. In old bouillon cultures (2 to 3 weeks), an appreciable deposit may be observed at the bottom of the tubes.

In bouillon to which glucose or lactose has been added, there is a freer growth of both of these bacilli, with the formation of fine gas bubbles at the surface of the glucose bouillon.

⁷ *Etiologia e patogenesi della febbre gialla. Il Policlinico*, 1897, iv -M., p. 397.

⁸ Hog-cholera. Bureau of Animal Industry, Washington, 1889, p. 64.

⁹ Wilder Quarter-century Book, p. 339. Ithaca, 1893.

We have not detected any definite odor in cultures of these bacilli.

In old bouillon cultures of *B. icteroides* and of the hog-cholera bacillus, many long rods are to be seen; some of these are swollen at one or both ends or in the middle (involution forms).

Gelatine.—In stick cultures of *B. icteroides* there is a slow growth along the entire line of puncture which appears as a delicate white line. The surface growth is seen as a thin, transparent layer, which shows little tendency to spread. This applies to cultures of *B. icteroides*, original, and *B. icteroides*, Havana.

This growth in gelatine characterizes also the hog-cholera bacillus, except that the surface growth is somewhat thicker, more irregular in outline, and shows more tendency to spread. In this respect, *B. icteroides*, Santiago, agrees with the hog-cholera bacillus.

In gelatine plates, after 24 hours at 20° C., the colonies of *B. icteroides* are invisible to the naked eye. As seen under the low power, they are round, colorless and finely granular. In crowded plates, some of the deep colonies without increasing much in size, become opaque and dark in color, appearing as round, almost black masses; others show a slight radial striation; still others a brownish tinge. Frequently the deep colonies present a dark centre surrounded by a lighter peripheral zone. Surface colonies show little tendency to spread. Under the low power, they are generally circular in outline, with sharply defined, smooth margin, although the latter may be irregular and indented. They present, as a rule, a central nucleus, surrounded by a colorless, granular zone which extends quite to the margin of the colony. In older colonies (4 to 10 days), the margin frequently becomes clearer and quite refractive, while the central part takes on a slight yellowish tinge.

The colony with central nucleus surrounded by a colorless, granular zone, with or without narrow, clear margin, may be taken as the typical surface colony of *B. icteroides*.

Surface colonies which are less often seen show a delicate radial or undulating striation extending from the central portion toward the periphery of the colony, constituting the so-called atypical colony. Sometimes this striation is made up of very numerous dark lines radiating from the centre to the periphery of the colony and giving the latter an appearance totally unlike the ordinary surface colony. Later these colonies may lose their striation entirely and show no distinctive markings. To the naked eye, well-developed surface colonies, by reflected light, are round, sharply defined, raised and glistening, and have been aptly compared to droplets of boiled starch or mucus. They present a delicate bluish translucence.

Both the deep and surface colonies of the hog-cholera bacillus in gelatine plates show the closest resemblance to those of *B. icteroides*, the rate of growth of *B. icteroides*, original, *B. icteroides*, Havana, and of hog-cholera No. 1 has been the same; that is, colonies are invisible to the eye after 4 hours at 20° C., whereas the colonies of *B. icteroides*, Santiago, and of hog-cholera No. 2, can just be distinguished after this interval as very minute white points. Under the low power, deep colonies of the hog-cholera bacillus are round, colorless and finely granular, or they may show a slightly brown color. With age, some of these colonies in the depth of the gelatine take on a dark color, and sometimes become quite black. We do not think that attention has heretofore been called to this darkening of deep colonies of the hog-cholera bacillus in gelatine. Surface colonies, as a rule, are smooth in outline, though they may be irregular and present the appearances already described for typical colonies of *B. icteroides*.

Colonies with undulating striation are also occasionally seen. We have not observed, however, in gelatine plates of the hog-cholera bacillus, those atypical surface colonies with dark, radial striation¹⁰ such as we have noted for *B. icteroides*. With this exception, we have observed no differences in colonies of these bacilli in gelatine plates.

Acid Potato Gelatine (natural acidity).—In stab and slant cultures there is a feeble development, after several days, all along the line of puncture or stroke. This applies to our several cultures of *B. icteroides* and the hog-cholera bacillus.

Acid Potato Gelatine with 1 per cent KI.—A very scant development also occurs in this medium with *B. icteroides* and the hog-cholera bacillus. The acidity of the medium used was such that 2 cc. of a decinormal sodium hydroxide solution were required to render 10 cc. neutral to litmus.

Agar-agar.—In stroke cultures on agar-slants grown at 35° to 37° C., *B. icteroides* forms a thin, moist, grayish-white layer. At 20° to 22° C., this growth is somewhat thicker and more convex.

The growth of the hog-cholera bacillus on agar-slants is quite similar to that of *B. icteroides*.

The Sanarelli bacillus recently obtained from cases of yellow fever, when grown as isolated colonies on agar-slants, first at 37° C., for 12 to

¹⁰ Since the above was written we have received from the Bureau of Animal Industry a culture of the hog-cholera bacillus recently isolated during an epidemic of hog-cholera at Fremont, Nebraska, which gives in gelatine plates atypical surface colonies with radial striation which cannot be distinguished from colonies of *Bacillus icteroides*.

24 hours, and afterwards at 20° to 28° C., will, according to its discoverer, show characteristic colonies which serve to distinguish it from all other bacteria. Under these conditions the colonies show two distinct zones, a central, flat, transparent area surrounded by a thick, prominent opaque zone, giving to the whole colony the appearance of a drop of sealing-wax.¹¹ As the colonies grow older, the external opaque zone becomes more transparent, and nearly disappears, while the central part remains as an opaque body embedded in it. In cultures, however, that have been repeatedly passed through animals, these characteristic colonies are less often seen.¹²

Novy¹³ states that this growth of the Sanarelli bacillus is always to be seen in isolated colonies grown on agar-slants, first at 39° for 20 to 24 hours, and afterward at 16° C. According to the same author, it is important that these temperatures should be observed, and that the medium should be distinctly alkaline.

In our experience, this characteristic appearance of colonies of *B. icteroides* on agar-slants grown as suggested by Sanarelli has not been constant. In our earlier experiments it was present in about 30 per cent of the cultures taken from the organs of inoculated guinea-pigs, rabbits and dogs; that is to say, a few isolated colonies in a tube presented this typical appearance. In our later cultures we have entirely failed to observe it. This failure to obtain the characteristic colony on agar-slants has also been noted by P. Foa¹⁴ and by de Lacerda and Ramos.¹⁵

The appearance of the thin central area, surrounded by a thicker opaque peripheral zone, is not, however, peculiar to *B. icteroides*, since we have occasionally observed the same appearance in isolated colonies of hog-cholera No. 1 grown on agar-slants, first at thermostat, and afterward at room temperatures. We have not observed any disappearance of the external thicker zone in colonies of the hog-cholera bacillus. Theobald Smith¹⁶ has already described and illustrated the peculiar appearance of concentric zones in isolated surface colonies of the hog-cholera bacillus grown in gelatine Esmarch rolls. These he considered "very likely due to changes of temperature in the laboratory, alternately retarding and augmenting the growth."

¹¹ *Policlinico*, pp. 428 and 429.

¹² *Ibid*, p. 431.

¹³ *Medical News*, 1898, lxxiii, p. 329.

¹⁴ Sul bacillo icterode (Sanarelli). *Giornale d. r. Accad. di med. di Torino*, 1898, 4. s., xlv, pp. 57 and 113.

¹⁵ Le bacille icteroïde et sa toxine. *Arch. de méd. expér.*, 1899, xi, p. 378.

¹⁶ Hog-cholera. Bureau of Animal Industry, p. 192 and Plate xi, Fig. 2. Washington, 1889.

As usually seen, isolated colonies on agar-slants of *B. icteroides* and of the hog-cholera bacillus, after 24 hours at 37° C., appear as thin, slightly convex or flattened discs, with smooth margins, and by reflected light present a waxy appearance. They measure 2 to 3 mm. in diameter and possess, as a rule, a faint bluish translucence.

Potato.—According to Sanarelli, *B. icteroides* appears as a moist, invisible growth on this medium. Our experience has shown that the appearance of the growth on potato is quite variable. When first transferred by us to potato, *B. icteroides* (original), grew as a moist, thin, brownish layer, with a tendency to spread over the surface of the medium. We have since had occasion to observe that upon different potatoes it may grow as a colorless, moist layer, or as a faint yellowish layer, or that it may show a decided brownish color.

The growth of the hog-cholera bacillus on potato in our hands has also shown decided differences on different potatoes. While, as a rule, it has presented itself as a thin, moist, yellowish or brownish layer, it has frequently appeared as a moist, invisible growth. On parallel potato cultures which we have recently made, our cultures of *B. icteroides* and those of the hog-cholera bacillus have all shown the same thin, moist, yellowish growth.

Plain Blood Serum.—*B. icteroides*, original, and *B. icteroides*, Havana, show only slight development on this medium. The growth is closely limited to the needle stroke and appears as a delicate, slightly raised, almost colorless layer. At the bottom of the stroke, near the surface of the fluid, there may occur a slight expansion in the growth, which is raised and of a dull grayish-white color. *B. icteroides*, Santiago, shows a much freer development all along the line of inoculation, and appears as a slightly raised, somewhat glistening grayish-white layer.

The appearance of the growth of hog-cholera Nos. 1 and 2 is quite similar to that of *B. icteroides*, Santiago, there being a free development all along the line of stroke.

On *Loeffler's blood serum* the same relative difference in development takes place as already indicated above for plain blood serum; that is to say, while the growth of *B. icteroides*, original, and of *icteroides*, Havana, is exceedingly limited, spreading but little along the line of stroke, that of *icteroides*, Santiago, and hog-cholera Nos. 1 and 2, is quite free all along the line of stroke, appearing as a transparent, flat, or as a slightly elevated grayish-white layer.

Isolated colonies of *B. icteroides*, original, and *B. icteroides*, Havana, on plain and glucose blood serum, appear as small, flat, transparent or slightly grayish colonies which do not exceed 0.5 to 1 mm. in diameter, while those of *B. icteroides*, Santiago, and hog-cholera bacillus Nos. 1

and 2 are larger, more elevated, of grayish-white color and measure from 1 to 3 mm. in diameter.

This relative difference in the growth of *B. icteroides* and the hog-cholera bacillus, as shown above, has been quite constant in our cultures on blood serum.

Milk.—This medium is not coagulated by either of these bacilli. The reaction of the milk becomes slightly acid at first, afterwards changing to neutral and later becoming strongly alkaline. The change to neutral and alkaline proceeds a little more rapidly with the bacillus of hog-cholera than with *B. icteroides*, original, and *B. icteroides*, Havana.

With *B. icteroides*, Santiago, the change from acid to alkaline corresponds with our cultures of the hog-cholera bacillus.

With our several cultures of Sanarelli's bacillus and the hog-cholera bacillus, the milk assumes a distinct opalescent appearance after 10 to 14 days at thermostat temperature. Later the medium becomes partly translucent.

Litmus milk is slightly decolorized by both organisms, taking on a somewhat muddy appearance. Later the original blue becomes restored and gradually deepened to an indigo blue.

Fermentation Tube.—The action of *B. icteroides* and of the hog-cholera bacillus upon the three sugars is the same. Both of these bacilli produce prompt and marked fermentation of glucose. In peptone bouillon containing 1 per cent glucose, there is a prompt appearance of gas during the first day, which is rapidly increased in amount during the second 24 hours, and reaches its maximum on the third or fourth day. The reaction of the bouillon becomes strongly acid. We have seen no appreciable difference in the quantity of gas produced by our cultures of *B. icteroides* and of the hog-cholera bacillus. The volume of gas represents about one-third of the closed branch of the fermentation tube. In composition this gas consists of CO_2 , 1 part; H , 2 parts.

No gas appears as the result of multiplication of either of these bacilli in peptone bouillon containing lactose or saccharose, provided means have been used to exclude all trace of muscle sugar. The reaction of the medium becomes distinctly alkaline.

Indol.—Tested by Kitasato's method, our cultures of *B. icteroides* and the hog-cholera bacillus give a faint indol reaction.

After discussing the general characters of the hog-cholera group of bacteria, Smith¹⁷ says:

¹⁷ Additional Investigations concerning Infectious Swine Diseases, by Theobald Smith and Veranus A. Moore. Bureau of Animal Industry, Bulletin No. 6, p. 27, Washington, 1894.

"If we attempt to sum up those characters which are to circumscribe the hog-cholera group of bacteria, we are at once confronted by the scarcity of common characters. Pathogenesis, though of great importance from the standpoint of pathology, is probably the last character acquired, and evidently the most variable and most readily lost. If we base the unity of this group on morphological and biological characters, we are likewise met by variations in size, absence of motility, variations in the appearance of the colonies. There are, however, certain underlying characters, as expressed by the behavior of these bacteria in bouillon containing dextrose, saccharose and lactose, which I think will serve as a very important group-character, differentiating such group sharply from the colon group. I would therefore suggest that, for the present, all bacteria whose size approximates that of this group, which do not liquefy gelatine, and whose fermentative properties are the same as those described for this group, should be arranged under it."

A comparison of the cultural characters already given indicates clearly that *Bacillus icteroides* should be placed in the hog-cholera group of bacteria.

Comparative Pathogenesis.

Both of the bacilli under consideration possess a considerable range of pathogenesis for animals. The hog-cholera bacillus is pathogenic, in varying degree, for mice, guinea-pigs, rabbits, pigeons, dogs and hogs. The lesions occurring in several of these animals are more or less characteristic and have been fully described by various observers. It will be of interest to compare the appearances found in the same animals when inoculated with *B. icteroides*.

Our observations with the latter bacillus have been generally confined to the inoculation of guinea-pigs, rabbits, pigeons, dogs and hogs. More recently, for comparative purposes, we have inoculated a few white mice subcutaneously with *B. icteroides* and have also fed the same animals with fluid cultures (0.1 cc.) of this bacillus. While the subcutaneous inoculation of mice is fatal after 2 to 4 days, we have observed that where infection has taken place through the digestive tract, death occurs with considerable regularity after about one week. The duration of the infection, and the gross lesions (enlargement of the spleen, areas of necrosis in the liver, congestion of the kidneys and of the mucosa of the small bowel) correspond closely with those already

recorded by Smith for mice inoculated with cultures of the hog-cholera bacillus.

Guinea-pigs and Rabbits.—These animals are quite susceptible to infection with *B. icteroides* or the hog-cholera bacillus, whether the culture is introduced beneath the skin, into the peritoneal cavity, into the trachea or intravenously. Infection may also be brought about by feeding moderate quantities of a fluid culture of either bacillus. As regards the degree of susceptibility, the rabbit has been found to succumb to smaller doses of both of these bacilli than the guinea-pig.

Smith¹⁸ succeeded in producing death in rabbits by inoculating them with $\frac{1}{1000000}$ cc. of a bouillon culture of the hog-cholera bacillus. We have found $\frac{1}{1000000}$ cc. of a 24-hour bouillon culture of *B. icteroides* sufficient to kill rabbits. Of two animals inoculated subcutaneously with this quantity, one died after 11, and the other after 12 days, with the usual lesions at autopsy.

In reference to the infection of guinea-pigs with *B. icteroides*, Sanarelli states that while the duration varies with the mode of inoculation, the disease follows a cyclical course which is not influenced, as a rule, by the quantity of culture inoculated subcutaneously. By the latter method guinea-pigs die on an average in from 5 to 8 days, the majority on the 7th. Exceptionally, they may die after 48 hours, or survive until 15 to 30 days. In further demonstration of this cyclical course of the disease, Sanarelli has killed inoculated guinea-pigs every 12 hours from the time of inoculation and has carefully studied cultures from the blood and organs, with the result that while a few colonies can be obtained from the spleen and liver after 12 to 24 hours, these organs are sterile from the 2d to the 5th day. On the 6th day, however, there is a sudden general invasion of the blood and organs by *B. icteroides* which is followed by the death of the animal on the 7th day.

While Bruschettini¹⁹ has confirmed Sanarelli's experience that the duration of the disease in guinea-pigs is not influenced by the quantity of the virus injected, provided the cultures are obtained from the guinea-pig or rabbit, he finds that cultures obtained from the blood of the dog will kill medium-sized guinea-pigs in 2 to 3 days, and sometimes within 24 hours.

We are not able to verify Sanarelli's statement that the dose injected subcutaneously, whether 0.1 cc. or 5 cc., has no influence on the duration of the malady. His statement appears to hold good provided the quantity does not exceed 0.5 cc. Under these circumstances, guinea-pigs

¹⁸ Hog-cholera, p. 71.

¹⁹ *Gazz. d. ospedali*, May, 1899, No. 64.

inoculated by us have died, as a rule, after 6 to 8 days; exceptionally, however, after 3 to 4 days. When the quantity injected was larger (1 cc. to 3 cc.), death has generally occurred at much shorter intervals, varying from 17 hours to 4 days. Exceptionally, guinea-pigs have survived doses of 3 cc. for a period of 7 days.

As to the sterility of cultures taken from the 2d to the 5th day after inoculation of guinea-pigs with *B. icteroides*, we can confirm the statement of Sanarelli; but we have obtained the same general results with guinea-pigs infected subcutaneously with small quantities of the hog-cholera bacillus; that is to say, while a few colonies may be obtained from the liver or spleen during the first 24 hours following the inoculation, cultures thereafter remain sterile until the day preceding the death of the animal—generally until the 5th to the 6th day after the injection. Since this behavior of the hog-cholera bacillus within the bodies of guinea-pigs corresponds with that recorded by Sanarelli for *B. icteroides*, it does not seem to us proper that any analogy should be drawn between this so-called cyclical course of the disease in the guinea-pig and the course of yellow fever in the human being. It would rather seem to indicate that after the introduction of a certain quantity of *B. icteroides* or the hog-cholera bacillus into the body of the guinea-pig, and the destruction of the few organisms that primarily invade the blood, an interval varying from 3 to 5 days is required before the natural resistance of the animal is overcome through the absorption of the toxic products of the micro-organisms multiplying at the point of injection, and that when this resistance is destroyed, the bacilli rapidly invade the blood and organs. In more susceptible guinea-pigs, the primary invasion is followed by rapid multiplication of the bacilli and death from septicæmia after 48 hours.

Turning now to the lesions produced in guinea-pigs and rabbits by the hog-cholera bacillus, these are found to be fairly constant and characteristic, namely, slight purulent infiltration at the site of inoculation, enlargement of the spleen and the presence of focal necroses in the liver. Fatty degeneration of the heart muscle is common. The duration of the disease depends upon the quantity and virulence of the culture inoculated, and varies from 4 to 12 days, the majority of the animals dying at about the end of one week.

In order to illustrate the lesions produced in these animals with *B. icteroides*, we submit protocols of some of our earlier experiments:

Exp. I.—Guinea-pig No. 456; weight 495 gm.; November 9, 1897, inoculated subcutaneously with 1 cc. of a 20-hour lactose bouillon culture of *B. icteroides* from blood of dog 443. Colonies from this dog on

agar-slant show characteristic "wax-seal" appearance. Death November 13, 1897, after 4 days. Weight after death, 318 gm.

Slight amount of purulent exudate at site of injection. Lymph-glands swollen and pale. Visceral peritoneum injected. No fluid in abdominal cavity. Small intestine of a rose-pink color. Spleen much swollen, soft, deep reddish-brown in color. Liver pale, numerous punctiform areas, yellowish-white in color, beneath its capsule and on section. Kidneys pale. Adrenals enlarged and congested. Upper lobe of right lung injected.

Cultures from blood, spleen, liver, kidney and urine give numerous colonies of *B. icteroides*.

Exp. III.—Guinea-pig No. 458; weight 680 gm.; November 9, 1897, 12.30 P. M., received under the skin 2 cc. of a 24-hour bouillon culture of *B. icteroides* from blood of dog No. 443. Death at 6.30 A. M., November 10, 1897, after 18 hours.

Considerable œdema at site of injection. Lymph-glands swollen. Parietal peritoneum injected. Spleen firm and small. Liver swollen and dark. Kidneys congested. Lungs normal.

Cultures from liver and spleen show a few colonies; other sources negative.

Exp. V.—Guinea-pig No. 461; weight 412 gm.; November 16, 1897, inoculated subcutaneously with 0.5 cc. glucose bouillon culture of *B. icteroides* from liver of guinea-pig 459. Death November 23, 1897, after 7 days. Weight after death, 268 gm.

No lesion at site of injection. Inguinal glands swollen and pale. Abdominal cavity contains about 2 cc. turbid fluid. Surface of liver and spleen covered with a thin grayish exudate. Spleen much swollen. Liver shows numerous small necroses, round, oblong, and of irregular shape; these are to be seen on the upper and under surface and on section. Kidneys swollen, congested throughout. Adrenals swollen and injected. Bladder filled with albuminous urine. Both lungs congested; pleural surfaces injected; small quantity of clear serum in pleural cavities.

Cultures from blood negative; from spleen, liver, bile, abdominal cavity, kidney and urine, positive. Colonies particularly numerous from spleen and abdominal cavity.

Exp. XI.—Guinea-pig No. 475; weight 420 gm.; November 27, 1897, received subcutaneously 0.3 cc. of a 24-hour lactose bouillon culture of *B. icteroides* from liver of dog 443. Death December 2, 1897, after 4 days and 20 hours.

Slight purulent exudate at site of injection. Inguinal and axillary glands swollen and pale. Both layers of peritoneum injected. Spleen

large and dark in color. Kidneys and adrenals swollen and much congested. Liver contains a small number of necroses. Lungs normal.

Cultures positive from blood, organs, bile and urine; very numerous colonies from spleen and bile.

Exp. I.—Rabbit; weight 2000 gm.; injected subcutaneously with 0.2 cc. of *B. icteroides*, original. Death after 7 days.

Lymph-glands enlarged. Splenic tumor. Liver large, congested and contains many small focal necroses. Kidneys swollen; on section, injected throughout.

Cultures positive from blood and organs.

Exp. II.—Rabbit; weight 1545 gm.; injected subcutaneously with 0.2 cc. of *B. icteroides*, original. Death after 8 days.

Some caseous purulent exudate at site of injection. Spleen swollen and dark. Liver normal. Kidneys congested. Lungs normal.

Cultures positive from blood and organs.

Exp. III.—Rabbit; weight 1707 gm.; received subcutaneously 0.2 cc. of a 24-hour culture of *B. icteroides*, original. Death after 8 days.

Splenic tumor. Numerous necroses in liver. A few hæmorrhagic areas scattered over duodenum. Injection of mucous membrane of upper part of small intestine. Small ecchymoses over the surface of both lungs.

Cultures from blood and organs positive.

It is seen from the foregoing protocols that the most prominent gross lesions in guinea-pigs and rabbits inoculated with *B. icteroides* are acute splenic tumor and multiple focal necroses in the liver. The latter have been quite constantly met in guinea-pigs when death has occurred after the 4th to the 6th day. In rabbits these necroses were sometimes absent. We have observed these necroses in animals inoculated with *B. icteroides*, original, and *B. icteroides*, Havana. Sanarelli does not record this lesion in guinea-pigs and rabbits, but states that the liver is always congested, except that, when the guinea-pig dies after many days, the liver presents a pale, gray, nutmeg appearance, and is evidently degenerated.

These necroses so frequently met by us, have been also observed by other investigators. De Lacerda and Ramos²⁰ record the finding of scattered yellow points ("*des points jaunes disséminés*") in the liver of one rabbit out of four inoculated with *B. icteroides*. They say nothing about the microscopic examination of these yellow points. Domenico della Rovere²¹ records as the result of his infection of guinea-pigs with *B. icteroides*, "as a new finding in the liver, foci of small cells situated in the midst of the lobules; the protoplasm of the liver cells appears very granular, the nuclei discolored and feebly stained, as well as the nuclei

²⁰ *Arch. de méd. expér.*, 1899, xi, p. 390.

²¹ Sul bacillo icteroide (Sanarelli). *Riforma medica*, 1898, xiv, pt. 3, p. 98.

of the cells lining the biliary canals and capillaries." Della Rovere also observed like foci of small cells situated within the hepatic lobules of rabbits inoculated with *B. icteroides*. Agramonte²² found these necroses in the livers of guinea-pigs inoculated with the culture of *B. icteroides* isolated by him from yellow-fever cadavers at Santiago, Cuba.

We will give briefly the results of *microscopic examination* of the several organs. For this purpose, tissues were hardened in 95 per cent alcohol, 5 per cent formalin, Orth's fluid and Flemming's osmic solution. The description applies to the tissues of both guinea-pigs and rabbits.

Frozen sections of the fresh organs were examined in normal salt solution for the detection of any fatty change. Fatty degeneration of the heart muscle was generally present. The gross change in the spleen, lymph-glands and adrenals appeared to be due to the large increase of the blood supply of these structures. No degenerative changes were observed except in the case of one guinea-pig that died on the 17th day after inoculation with *B. icteroides*, Havana. Here extensive foci were found in both the follicles and splenic pulp. The cells in these areas had been converted into a granular detritus, in the midst of which abundant minute nuclear fragments were to be seen. A moderate number of polymorphonuclear leucocytes were invading these areas from the margin.

In the kidney, both fatty and parenchymatous degenerations of the tubules of the labyrinth were observed; the former was slight, the latter more marked. The general blood supply of the organ was increased, as shown in the marked dilatation of the glomerular capillaries and of the intertubular vessels. An amorphous exudate and a few red corpuscles were frequently found within Bowman's capsules. Sometimes there was apparent loss of the glomerular epithelium; no interstitial changes were present. The lesions in the kidney did not differ from those seen in other acute experimental infections.

The liver presents, in a majority of the cases, more marked changes. In addition to the congestion which is generally present, there is sometimes seen slight fatty metamorphosis. This was never marked on microscopic examination of frozen sections, although the color of the organ at autopsy at times seemed to indicate this change. In the majority of cases, in both guinea-pigs and rabbits, there was an increase in the number of polymorphonuclear leucocytes within the hepatic capillaries. In some instances the capillaries were so crowded and distended with these leucocytes as to constitute veritable thrombi. There did not ap-

²² The *Bacillus icteroides* (Sanarelli) and *Bacillus X* (Sternberg). By Geo. M. Sternberg. *Centralbl. f. Bakteriologie*, 1899, xxv, p. 659.

pear to be any relation between these collections of leucocytes and the areas of necrosis, the latter presenting the most striking feature to be seen in the liver (fig. 1, Plate XIX). These foci varied much in size. Sometimes only a few cells were affected, but generally a considerable part of the lobule was involved. At times an entire lobule and adjacent parts of several lobules had undergone the necrotic change. Various stages in this process of coagulative necrosis were seen. Areas were observed in which the protoplasm of the cells stained brightly with eosin and appeared more granular than usual, while the nuclei remained visible, although paler than normal. In other areas, the nuclei of the more centrally placed cells had quite disappeared, while those of the peripheral cells had only suffered a slight alteration in their capacity to take nuclear stains. In still other areas, the cells had become hyaline and refractive, and the nuclei had entirely disappeared without leaving behind any nuclear detritus within the cells. The number of leucocytes present in these areas varied considerably. At times none were to be seen; but, as a rule, many were found collected about the margin, or were contained within the capillaries of the outer zone of the area. Again, they had penetrated in large numbers to the centre and had there undergone fragmentation. In animals that died after considerable periods (9 to 17 days), foci were observed in which the liver cells had completely disappeared and in whose place there were to be seen, in addition to a few polymorphonuclear leucocytes, many cells with large epithelioid nuclei, and fewer cells with small, round, deeply staining nuclei.

The foregoing description applies equally, in our experience, to the livers of rabbits and guinea-pigs inoculated with the hog-cholera bacillus.

Dogs.—The clinical picture brought about in dogs by the intravenous injection of *B. icteroides* is strikingly reproduced by the inoculation of these animals with the hog-cholera bacillus. In the course of our experiments we have inoculated eight dogs with *B. icteroides*. Of these 5 have died and 3 recovered. We give protocols of some of the fatal cases.

Exp. I.—Dog No. 443; weight $10\frac{1}{2}$ lbs. September 24, 1897, 1.45 P. M., inoculated intravenously with 5 cc. of a 24-hour lactose bouillon culture of *B. icteroides*, original. Temperature before inoculation 102° F. Within 15 minutes, frothy, tenacious saliva was observed about the mouth; animal very restless. 2.20 P. M., loose action with tenesmus, followed by vomiting of a frothy, gray fluid; the act of vomiting was several times repeated with much retching. 2.45 P. M., slimy stool mixed with blood. 4.20 P. M., temperature 105.2° .

September 25, 9 A. M., temperature 104° ; 3 P. M., temperature 105.8° . Animal has had several black, tarry-looking stools; remains quiet and refuses food. This elevation of temperature continued until October 1, when it became subnormal, falling to 98° F. at 1.40 P. M., October 2, and to 93.4° F. at 10.20 A. M., October 3. Death at 6 P. M., October 3, after 9 days.

Autopsy at 2.30 P. M., October 4, the body in the meanwhile having been kept in ice-chest. Subcutaneous tissues dry. Inguinal lymph-glands swollen and injected. Stomach and intestine pale. A few hæmorrhagic areas scattered over surface of ileum. Mesenteric glands swollen and moderately congested. No fluid in abdominal cavity. Spleen firm, slightly enlarged. Liver of a generally pale color; outlines of lobules distinct. Kidneys swollen, pale; small, distinct hæmorrhagic points beneath capsule; on section, cortex is thickened and cloudy in appearance; pyramids slightly injected. Bladder contracted; contains a small amount of albuminous urine. Mucous membrane of the stomach and intestine pale. Lungs normal. Right cardiac cavities distended with fluid blood.

Cultures from blood, spleen, liver, bile, urine and kidney give colonies of *B. icteroides*.

Microscopic examination of frozen sections shows moderate fatty degeneration of the liver, which is confined to the peripheral part of the lobules. Hardened sections show, in addition, small areas of coagulative necrosis.

Exp. II.—Dog No. 465; weight $16\frac{1}{2}$ lbs. November 16, 1897, at 3 P. M., injected intravenously with 5 cc. of a 24-hour bouillon culture of *B. icteroides*, original. Temperature before inoculation 102.6° F. 3.27 P. M. vomited food, followed by micturition and evacuation of bowel with tenesmus. Acts of vomiting and defecation repeated several times during the next 2 hours; animal considerably prostrated. 4.10 P. M., temperature 102.6° . November 17, 9.40 A. M., temperature 103.4° ; 4 P. M., 104° ; animal refuses food; appears much dejected. Temperature remained elevated until November 22, at which time the dog's condition was much improved.

November 30, at 2 P. M., the animal was a second time injected with 5 cc. of a 24-hour culture of *B. icteroides* from the blood of dog 443. When returned to its cage, the dog was profoundly prostrated, and died 28 minutes later.

Autopsy 30 minutes after death. An abundance of subcutaneous fat. Spleen small and light-red in color. Liver dark and congested; blood flows freely from cut surface. Kidneys swollen, cortex pale, pyramids injected. Bladder firmly contracted. Mucous membrane of stomach

pale. Mucous membrane of small intestine deeply congested from the pylorus to the ileocaecal valve. Lungs normal.

Cultures from liver and spleen, positive; blood, bile, abdominal cavity, kidney and urine, negative.

Frozen sections of the liver and sections hardened in Flemming's solution show slight fatty degeneration.

Exp. III.—Young dog No. 504; weight 12½ lbs. December 13, 1897, 11.20 A. M., injected intravenously with 2.5 cc. of a 24-hour glucose bouillon culture of *B. icteroides* from liver of dog 443. Temperature before injection 102.2° F. Within the next few hours following the injection there were repeated acts of vomiting and defecation with much tenesmus, followed by marked prostration. 4 P. M., temperature 105.4°.

December 14, 9.20 A. M., temperature 103.6°; 4 P. M., 106.2°; animal has frequent loose stools mixed with mucus and blood. The animal remained in this condition and died at 8 A. M., December 22, 1897. Weight after death 9½ lbs.

Autopsy 3 hours after death. Lymph-glands much swollen and injected. No fluid in abdominal cavity. Spleen enlarged, soft, dark red in color. Liver paler in color than normal; outlines of lobules distinct; peripheries pale; central veins congested, surrounded by lighter areas; cut surface presents the same appearance. Kidneys swollen; cortex swollen, cloudy. Urinary bladder partly contracted, containing albuminous urine. Stomach contains several ounces of a dark green, brownish fluid; mucous membrane pale. The same pallor applies to the whole mucous membrane of small intestine. Some congestion of the rectum, which contains a small quantity of dark grumous-looking fluid. Lungs normal. Increased fluid in pericardial sac; numerous punctate hæmorrhages beneath visceral pericardium; all cavities of heart distended with dark fluid blood; many punctate hæmorrhages beneath endocardium of both ventricles; myocardium pale.

Cultures from blood, spleen, liver, bile, kidney and urine gave colonies of *B. icteroides*.

Frozen sections of the liver show marked fatty degeneration, the lobules being involved throughout; the hepatic cells are filled with fine and medium-sized oil drops; the cells lining the bile ducts also contain many fine oil drops. Frozen sections of the kidney show advanced cloudy swelling, with slight fatty change in the secreting epithelium.

In two other dogs injected intravenously with 5 cc. of a culture of *B. icteroides*, death occurred after 52 and 16 hours respectively, preceded by the same clinical symptoms. There was present intense hæmorrhagic gastro-enteritis, but no fatty change in the liver, although focal necroses were present in this organ.

For comparative purposes we have also inoculated dogs with the bacillus of hog-cholera. Of these 5 have died at various intervals following the inoculation, and 5 have recovered.

We submit protocols:

Exp. I.—Dog No. 595, weight $12\frac{1}{2}$ lbs. May 16, 1898, 1.15 P. M., received intravenously 5 cc. of a 24-hour bouillon culture of the hog-cholera bacillus. Temperature before inoculation 102.6° . Within an hour animal appeared much affected. Vomited at 2.05 and 2.40 P. M. Frequent acts of micturition and defecation during the afternoon. The stools consisted of watery fluid mixed with mucus and blood. 5 P. M., temperature 103.2° . At this time animal is completely prostrated. Death at 7.30 A. M., May 17.

Abdominal cavity contains a small quantity of a blood-stained serum. Peritoneum injected throughout. Scattered points of hæmorrhage over large and small intestine. Spleen swollen and congested, firm. Liver dark in color and deeply congested. Bladder firmly contracted. Kidney swollen; cortex pale, pyramids injected. Stomach contains dark fluid blood; its mucous membrane is uniformly and deeply congested. Small intestine also contains fluid blood; its mucous membrane is deeply injected and hæmorrhagic from pylorus to ileocæcal valve. Thoracic organs normal.

Cultures from blood, liver and spleen positive.

Frozen sections show cloudy swelling but no fatty change in either liver or kidney.

Exp. V.—Dog No. 1060; weight 14 lbs. June 2, 1899, 2.40 P. M., received intravenously 5 cc. of a 24-hour bouillon culture of the hog-cholera bacillus. Temperature before inoculation 100° F. The symptoms as already described for the preceding animal appeared within an hour. Spells of vomiting, micturition and defecation were especially frequent in this animal. June 3, 9.30 A. M., temperature 101.8° ; 4 P. M., 101.9° . Dog refuses food; appears to be very sick. June 4, 10.30 A. M., temperature 105.4° ; 4 P. M., 105.8° . This elevated temperature continued until June 8, after which date fever subsided and animal gradually recovered its strength.

June 15, 1899, 3.40 P. M., again injected in the ear-vein with 5 cc. of a 48-hour culture of the hog-cholera bacillus, followed by death at the end of 45 minutes.

Autopsy 18 hours after death, body having been kept in an ice-box in the meanwhile. About 25 cc. of blood-stained serum in the abdominal cavity. Omentum and both layers of peritoneum congested. Spleen pale and flaccid, not enlarged. Liver firm, congested. Bladder contracted; its mucous membrane distinctly congested. Kidneys swollen

and much congested. Gastric mucosa intensely injected throughout, swollen and hæmorrhagic. Mucous membrane of small intestine is swollen and of a raspberry-jam color from pylorus to the ileo-cæcal valve. That of the large bowel is less congested. Lungs normal. All cavities of the heart distended with dark fluid blood; numerous subendocardial ecchymoses in left ventricle.

Cultures from the blood, sterile; from the liver, kidney and spleen, positive.

Frozen sections of the liver show moderate fatty degeneration of the hepatic cells.

Exp. IX.—Dog No. 1109; weight 15 lbs. This animal received intravenously three injections of the hog-cholera bacillus, as follows:

August 22, 1899, 8 cc., September 6, 1899, 25 cc., November 4, 1899, 25 cc. of a bouillon culture. After each injection there were the same clinical symptoms already described in previous dogs, followed by marked fever. Death November 18, 14 days after last injection.

Autopsy 6 hours after death. Spleen somewhat swollen, firm and moderately injected. Liver of paler color than normal; markings indistinct. Kidneys swollen, cortex thickened and cloudy. No hæmorrhagic lesions in stomach or intestinal canal. Bladder partly distended with albuminous urine. Lungs normal.

Cultures from blood, sterile; spleen, liver and kidneys, positive.

Frozen sections of the liver show fatty degeneration. Hardened sections show small areas of coagulative necrosis.

In two additional experiments with dogs, 5 cc. of a bouillon culture of hog-cholera bacillus injected intravenously, brought about death, in the one case after 10 hours, and in the other at the end of 21 days, the animal being extremely emaciated. In the former, hæmorrhagic lesions were marked in stomach and small intestine. In neither case was there any fatty degeneration of the hepatic cells.

In our experiments with dogs we have recovered *B. icteroides* or the hog-cholera bacillus respectively in pure culture. In no case of either series of experiments was there a mixed infection.

An examination of the foregoing protocols of dogs inoculated with the hog-cholera bacillus, will serve to demonstrate that the same clinical picture is seen in these animals as in those inoculated with Sanarelli's bacillus. We have constantly observed repeated vomiting, increased action of the bowels with tenesmus, frequent micturition, and pronounced prostration with fever. These symptoms have appeared within an hour or less after the injection, and in two instances were followed by death after 10 and 18 hours respectively. At autopsy, intense hæmorrhagic

gastro-enteritis was present as seen in dogs that died within a short interval after injection with *B. icteroides*.

Thus, in dogs inoculated with the hog-cholera bacillus, we have been able to reproduce a part of the clinical and anatomical picture which Sanarelli considers analogous to that seen in yellow fever in the human being. It is important to observe, however, that these symptoms and lesions in the dog are not peculiar to either *B. icteroides* or the hog-cholera bacillus, since we have fully reproduced them in dogs by the intravenous injection of a member of the colon group (*Bacillus X*, Sternberg). There are probably other bacteria that would bring about the same symptoms and lesions in dogs.

As regards the fatty degeneration of the liver, the second part of the anatomical picture upon which Sanarelli lays considerable importance, we have met with this in less degree in dogs injected with the hog-cholera bacillus. Although it was present in 2 out of 5 autopsies, the degree of fatty degeneration was not marked in any of these. On the other hand, in only 1 of 5 dogs in which a fatal infection was produced with *B. icteroides* was this change present in sufficient degree to constitute an important pathological lesion. In 2 other dogs injected with Sanarelli's bacillus, there was a moderate degree of fatty degeneration, just as there was in 2 of the dogs injected with the hog-cholera bacillus. Thus, in the young dog of the icteroides series in which fatty degeneration of the liver was a prominent feature, this did not reach that extent or degree seen in the liver of yellow fever. Moreover, there was a conspicuous absence of cellular necrosis, always so prominent a feature in the liver of the latter disease.

The percentage of cases in which we have observed fatty degeneration in the liver of dogs inoculated with *B. icteroides* (60 per cent), while slightly less than that observed by Sanarelli²³ (71.3 per cent), is in excess of that reported by de Lacerda and Ramos²⁴ (50 per cent). We have been unable to find detailed reports of observations by other workers.

In connection with the production of fatty degeneration of the liver of dogs injected with *B. icteroides*, we observe that P. Foa,²⁵ having produced in the liver of one dog inoculated with *B. icteroides* extreme steatosis in all respects, as he states, similar to that found in the liver of yellow fever, failed to encounter again this change in a series of dogs (the number is not stated), although these died after varying intervals and in a marasmic condition. We have already shown in a pre-

²³ *Polielinico*, pp. 445, 449.

²⁴ *Arch. de méd. expér.*, 1899, xi, p. 390.

²⁵ *Giornale d. r. Accad. di med. di Torino*, 1889, 4. s., xlv, p. 115.

vious paper²⁶ that the hog-cholera bacillus, in larger doses several times repeated, may cause intense fatty degeneration of the dog's liver.

That *B. icteroides* may fail to bring about fatty degeneration even in the liver of human beings, is shown by the case of Private Patrick Smith, 8th Infantry, who died in Havana on the 9th day of his illness (Case No. 7 in Wasdin and Geddings' Report).²⁷ *B. icteroides* was isolated from the blood of this case 4 days before death by these observers, and again at autopsy by Agramonte.²⁸ The liver, however, neither at autopsy nor upon careful microscopic examination showed any trace of fatty degeneration, but only limited areas of necrosis, invaded by leucocytes (Fig. 2, Plate XIX). Although there was no intestinal ulceration present in this case, Agramonte isolated at autopsy also the typhoid bacillus, a culture of which we have been permitted to examine and verify. In this case, therefore, *B. icteroides*, which was present in the blood four days before death, did not produce that fatty degeneration of the liver which is so constant in yellow fever (Fig. 3, Plate XIX).

The focal necroses which we have observed in the livers of dogs inoculated with these two bacilli have been reported also by Foa²⁹ in the liver of a dog inoculated with Sanarelli's bacillus.

Pigeons.—Bruschettini³⁰ states that a small quantity of a virulent culture of *B. icteroides* when injected into the breast muscle of the pigeon causes a fatal infection. In his hands the injection of 2 cc. of the culture killed one pigeon after 12 days. By inoculating successive pigeons with blood obtained at autopsy, he was able to produce death in 4 or 5 days. Besides swelling and discoloration at the site of the inoculation, there was enlargement of the spleen and diffuse gastro-enteritis. Microscopically, the breast muscle was the seat of marked fatty degeneration.

We have injected a few pigeons with *B. icteroides* and found them tolerably resistant to this organism. Less than 3 cc. of a 24-hour bouillon culture injected into the pectoral muscle has not sufficed to bring about death. 3 cc. generally cause death after 5 days, but have occasionally failed to kill. We have found marked swelling at the point of injection, together with widespread necrosis of the breast muscle interspersed with areas of hæmorrhage. The spleen was enlarged, and the mucosa of the small intestine injected. Cultures were positive from the wound, blood and organs.

²⁶ *Medical News*, 1899, lxxv, p. 321.

²⁷ Report on the Cause of Yellow Fever. U. S. Marine Hospital Service, Washington, 1899.

²⁸ *Centralbl. f. Bacteriologie*, 1899, xxv, p. 661.

²⁹ *Op. cit.*, p. 115.

³⁰ *Op. cit.*, p. 698.

Microscopically, the muscle fibres are found to be much swollen and to have undergone complete necrosis.

The greater resistance shown by pigeons, as compared with the smaller laboratory animals, to infection with *B. icteroides* is also manifested toward the hog-cholera bacillus. The widespread necrosis at the site of inoculation is common to both of these bacilli.

Swine.—Our experiments with these animals have consisted in feeding them with pure cultures of *B. icteroides*, as well as with the viscera of infected pigs. We have also exposed young pigs to natural infection by confining them in boxes in which other swine had died after being fed with Sanarelli's bacillus.

Exp. I.—Young pig No. 919; weight $16\frac{3}{4}$ lbs. March 6, 1899, 1 P. M., was fed 25 cc. of a 24-hour plain bouillon culture of *B. icteroides*, original, which had passed through one guinea-pig. The culture was fed in a pint of milk. Rectal temperature prior to feeding, 102.4° F. The animal appeared ill on the following day, ate but little and persisted in lying down. Temperature, 9.30 A. M., 105.6° ; 3 P. M., 105.6° . March 8, temperature 9 A. M., 104.2° ; 3 P. M., 104° . Animal refuses food and shows weakness of the hind extremities. March 9, temperature 9.30 A. M., 102.6° ; 3 P. M., 103.4° . March 10, temperature 9.30 A. M., 101.8° ; 3 P. M., 102° . Slight diarrhœa appears on this date. March 11, temperature 9.30 A. M., 95.5° ; 3.30 P. M., 102.8° . Slight diarrhœa continues. Animal has refused all food for 2 days. Death 6 P. M., March 11.

Autopsy 10.30 A. M., March 12, the pig having been kept on ice in the meanwhile. No lesions on lips or in mouth. Marked injection of subcutaneous tissues. Axillary glands swollen. Abdominal cavity contains about 20 cc. of blood-stained serum. Both layers of peritoneum congested. Omental vessels, as well as mesenteric, are much engorged. Mesenteric glands swollen and injected. Spleen swollen, soft, congested. Liver, attached to diaphragm by moderately firm adhesions, is of a dark color, and on cut surface shows a number of small, irregular, yellowish, bile-stained areas. Gall-bladder moderately distended with dark, greenish, thick bile; its mucous membrane deeply injected. Kidneys swollen, cortex pale, pyramids congested. Lungs normal; no fluid in pleural sacs. Stomach normal. In the lower two-thirds of the ileum there were a number of circumscribed areas of diphtheritic inflammation. The large intestine is the seat of extensive diphtheritic inflammation beginning a few inches below the ileocæcal valve; its mucous membrane congested, swollen and covered with an adherent fibrinous exudate. This condition extends about 18 inches down the gut. Below this point there

are numerous circumscribed superficial erosions until the rectum is reached, which shows no lesions.

Cultures from the blood, liver, spleen, kidney and mesenteric gland are positive.

Exp. II.—Pig No. 927; weight 14 lbs. March 12, 1899, was fed a portion of the viscera obtained from pig 919. Temperature before feeding was 103.5°, 12 M. March 15, animal shows decided symptoms of sickness. Temperature 9 A. M., 104.6°; 4 P. M., 106.4°. Refuses all food to-day. The fever, with lack of appetite, continued until March 18. From this time animal improved and fever subsided. Was killed March 31.

No fluid in abdominal cavity. Spleen slightly congested and firm. Liver normal in appearance. In the lower two feet of the ileum the mucosa is uniformly and deeply injected. The large intestine, beginning with the cæcum, contains a number of round or irregular healing ulcers. Some of the ulcers show a slightly raised margin and appear to be in various stages of cicatrization.

Cultures from blood, organs and mesenteric glands, negative.

As in these two experiments the animals were confined in a room where a dog had died a few weeks previously from the intravenous injection of the hog-cholera bacillus, we procured, as a matter of precaution, a second-story, well-isolated room, with cemented floor in which no animals had ever been inoculated. Four new wooden boxes were procured, and in each of these a young pig was placed on April 6, 1899. These animals had been purchased in open market and were fat and healthy in appearance.

As a result of feeding these pigs with cultures of *B. icteroides*, the animals promptly sickened and died from the 6th to the 12th day after inoculation. We give protocols of some of these experiments:

Exp. III.—Young pig No. 976; weight 15 lbs. April 8, 1899, 3 P. M., fed 25 cc. of a bouillon culture of *B. icteroides*, original, which had passed through one guinea-pig. Temperature before inoculation, 102° F. April 10 there was diarrhœa with thin yellow stools of pea-soup consistency. Temperature 11 A. M., 106.2°; 4 P. M., 107°. April 14, fever and diarrhœa continue; animal shows distinct weakness in hind extremities, standing with back arched. Temperature 9 A. M., 101.5°; 4 P. M., 104°. From this date animal grew weaker, refused all food, and died 2.15 P. M. April 17, nine days after feeding. Weight after death 9½ lbs.

Autopsy one hour after death. No lesions in mouth or on lips. Some injection of subcutaneous tissues. Inguinal glands enlarged. No fluid in abdominal cavity. Small intestine generally congested. Spleen pale and firm, not swollen. Liver of dark-red color. Stomach contains

several somewhat curved linear ulcers with hæmorrhagic base. Small area of hæmorrhage beneath peritoneal coat of duodenum. A second similar area over surface of ileum. Mesenteric glands swollen. Mucosa of small intestine congested throughout and swollen. The cæcum and large intestine are the seat of marked diphtheritis with abundant yellowish-gray exudate which covers the mucous membrane as a lining; there are also distinct, irregular ulcers whose surfaces are covered with bile-stained necrotic material. These ulcers are also found in the rectum. Lungs normal.

Cultures from abdominal cavity, blood, bile, liver, kidney, urine and spleen, sterile; from mesenteric gland, positive.

Exp. IV.—Young pig No. 977; weight $12\frac{1}{2}$ lbs. April 8, 1899, 3 P. M., fed 15 cc. of a 24-hour bouillon culture of *B. icteroides*, original, which had passed through one guinea-pig. Temperature before feeding, 103° . April 10, the animal eats but little; has thin, pea-soup-looking stools. Temperature 11 A. M., 103.5° ; 4 P. M., 105° . This diarrhœa and fever continued, followed by increasing weakness and death on April 14, 1899, after 6 days.

At autopsy there are three ulcers on the mucous membrane of the upper lip. Two are very small and undergoing cicatrization. The third is nearly circular in outline, depressed, and with margins brightly injected. It measures 7 x 8 mm. A similar ulcer with necrotic centre is present on the lower lip. Inguinal glands swollen, pale. Parietal and visceral peritoneum generally injected. Spleen small. Liver injected. Kidneys show cloudy cortex. Mesenteric glands swollen and congested. Mucosa of stomach much congested over the greater curvature. Several small ulcers measuring 2 to 3 mm. in diameter are found in this region. Each ulcer bears a whitish superficial slough. Upon scraping this away, a small crater-like excavation is exposed. The mucosa of the small intestine is swollen and congested throughout. In the lower six feet of the ileum there are numerous small ulcers with superficial sloughs. There is also seen a Peyer's patch with thickened margins and excavated centre, the whole being covered with a thick, bile-stained necrotic material. For a distance of two feet above the ileocæcal valve, the entire surface of the mucous membrane has undergone necrosis. There is also diffuse necrosis of the mucous membrane of the cæcum and of the large bowel for a distance of about two feet (Fig. A). Below this point there are a number of discrete ulcers, the intervening mucous membrane being swollen and injected. These ulcers are less numerous in the rectum.

Cultures from the blood and spleen, negative; from liver and mesenteric glands, positive.

Exp. VI.—Young pig No. 979; weight 14 lbs. April 10, 1899, fed 15 cc. of a 24-hour bouillon culture of *B. icteroides*, original, passed through one guinea-pig. The same clinical picture was seen as in preceding experiments, viz., diarrhœa, fever beginning on April 12, and this followed by weakness, emaciation and loss of strength in hind legs.



FIG. A.

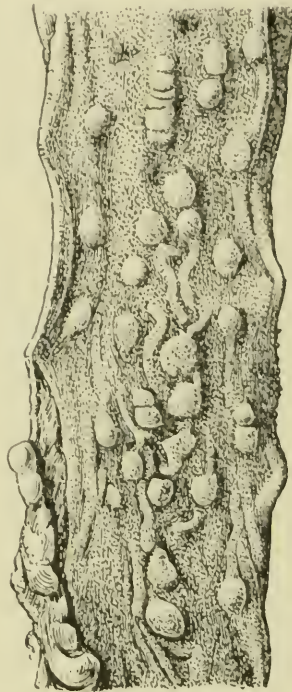


FIG. B.

FIG. A.—Colon of hog showing “cork-lining” appearance due to excessive thickening and necrosis of the mucous membrane. Death on 6th day after ingestion of 15 cc. of a bouillon culture of *B. icteroides*.

FIG. B.—Circumscribed nodular thickening in submucosa of large intestine of hog. Death on 12th day following ingestion of 15 cc. of a bouillon culture of *B. icteroides*.

Subnormal temperature observed on April 18 and continued till death, on April 22, 1899, after 12 days.

No lesions on lips or in the mouth. Inguinal and cervical glands swollen. Both layers of peritoneum injected. Mesenteric glands swollen. Spleen small and firm. Liver congested. There is a distinct grayish diphtheritic exudate upon the mucous membrane of the œsoph-

agus, which becomes more marked at the lower end. A circumscribed patch of diphtheritis is found near the cardiac end of the stomach and several small, shallow ulcers with hæmorrhagic bases along the lesser curvature. A few inches below the pyloric orifice there is a circular, raised, button-like mass, measuring 7 mm. in diameter by 3 mm. in thickness. Over this mass, the mucosa is intact. It projects slightly on the peritoneal surface. The mucosa in the lower ileum is swollen and congested. Small superficial erosions are found in this part of the gut. Two small ulcers are situated on the ileocaecal valve. The cæcum contains a few ulcers covered with a grayish, dirty-looking diphtheritic exudate. Areas of circumscribed diphtheritis are also present. The mucosa in the upper part of the bowel is swollen and congested. Commencing at a point about 12 inches below the cæcum, the bowel is studded with numerous grayish, firm, elevated nodular masses, varying

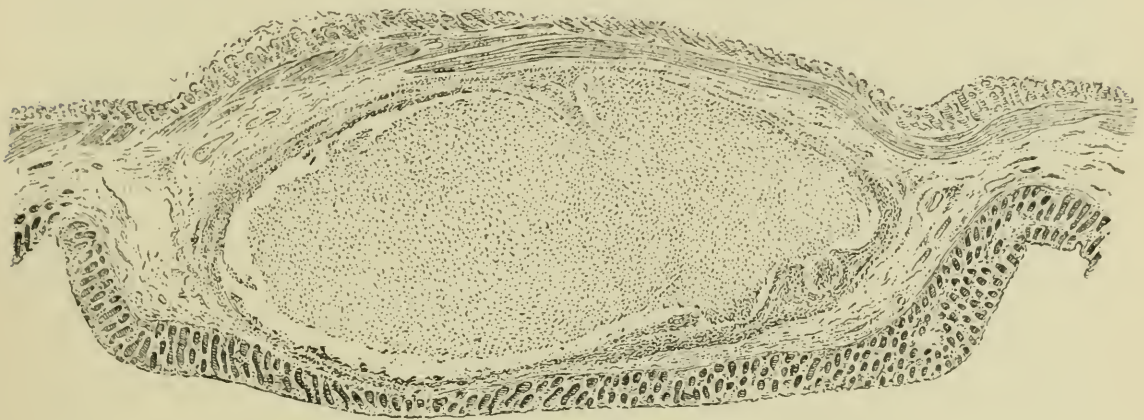


FIG. C.

FIG. C.—Section of one of the nodules of Fig. B, showing central necrosis. $\times 24$.

from 4 to 7 mm. in diameter. Over most of these the mucosa appears to be intact, but a few show a central necrosis. These nodules are absent from the rectum. They appear to be situated in the submucosa and project slightly on the peritoneal surface of the bowel (see Figs. B and C).

Cultures from blood, spleen, liver and kidney, sterile; from mesenteric gland, positive.

It is important to note that in Experiments I, III, IV and V (the last protocol we have omitted), we obtained in pure culture a small actively motile bacillus, which upon being transferred to Loeffler's blood serum, showed the same sparse growth as has already been described for *B. icteroides*, original. The culture obtained in Experiment VI was accidentally destroyed before it could be further examined. In Experiment VII, however, (whose protocol we do not give), in addition to the acute lesions of the hæmorrhagic type of hog-cholera, there were present

on microscopical examination of sections of the colon, well-defined necrotic areas in the submucosa. Death occurred on the 11th day after feeding 15 cc. of a bouillon culture of *B. icteroides*, original. From the blood, spleen, liver, kidney and mesenteric gland, there was obtained, in pure culture, a bacillus which corresponded in morphological and biological characters to *B. icteroides*, original. One pig, Experiment VIII, resisted repeated feedings of *B. icteroides*, original.

In order to ascertain whether the domestic pig could be naturally infected with *B. icteroides* when placed in conditions favorable for acquiring the infection, the following experiment was made:

Exp. IX.—Young pig No. 995; weight 10 lbs. Lips and mouth free of lesions. April 25, 1899, was placed in the box in which pig 978 had died on April 17, 1899. Temperature before being placed in the box, 103°. The animal ate its food heartily and showed no sign of sickness nor fever until May 3, 1899, nine days after exposure. On this date its rectal temperature at 4 P. M. was 106.3°. From this time the pig appeared to be sick, ate but little and became emaciated and weak. Fever varying from 105° A. M. to 106.1° P. M., continued till May 9; there was no diarrhœa observed at any time. May 11, temperature subnormal. Death May 12, 1899, after 17 days.

Autopsy 6 hours after death. The skin of all of the extremities shows a bright flush. There are several small ulcers undergoing cicatrization on both lips. Lymph-glands moderately enlarged. Mesenteric vessels congested; mesenteric glands swollen; through the wall of the large intestine, numerous pale, circular, opaque areas are visible; these measure from 2 to 6 mm. in diameter. Spleen enlarged and dark in color. Liver congested. Kidneys swollen; cortex thickened and cloudy; pyramids injected. Near the cardiac orifice of the stomach there are several small roundish ulcers with raised grayish sloughs. The fundus of the stomach is deeply injected and of a raspberry-jam color. Mucosa of lower ileum swollen and hyperæmic. The cæcum is studded with small ulcers bearing yellowish or grayish sloughs. The valve is surrounded by similar ulcers which encroach upon its base. These ulcers are also present in the large bowel, together with numerous roundish areas of superficial necroses. These are also found in the rectum. Thoracic organs, normal.

Cultures from blood, liver, kidney, spleen and mesenteric gland, positive.

Of three other pigs exposed to natural infection, under like conditions, two died after 27 and 40 days, respectively, and one remained unaffected.

We have also succeeded in producing a fatal infection in two pigs by feeding the viscera of pigs that have recently died from acute infection with *B. icteroides*, death occurring in these cases after 15 and 16 days

respectively. The lesions in these various animals corresponded with those already described.

From the mesenteric glands of each of these pigs we have isolated in pure culture a bacillus which agreed in its sparse development on blood-serum with *B. icteroides*.

The foregoing experiments with the domestic pig were made with *B. icteroides*, original, which had been passed through the body of one guinea-pig.

In order to ascertain whether the cultures of this bacillus which had been obtained from other sources, would bring about a fatal infection in young swine, we made the following experiments:

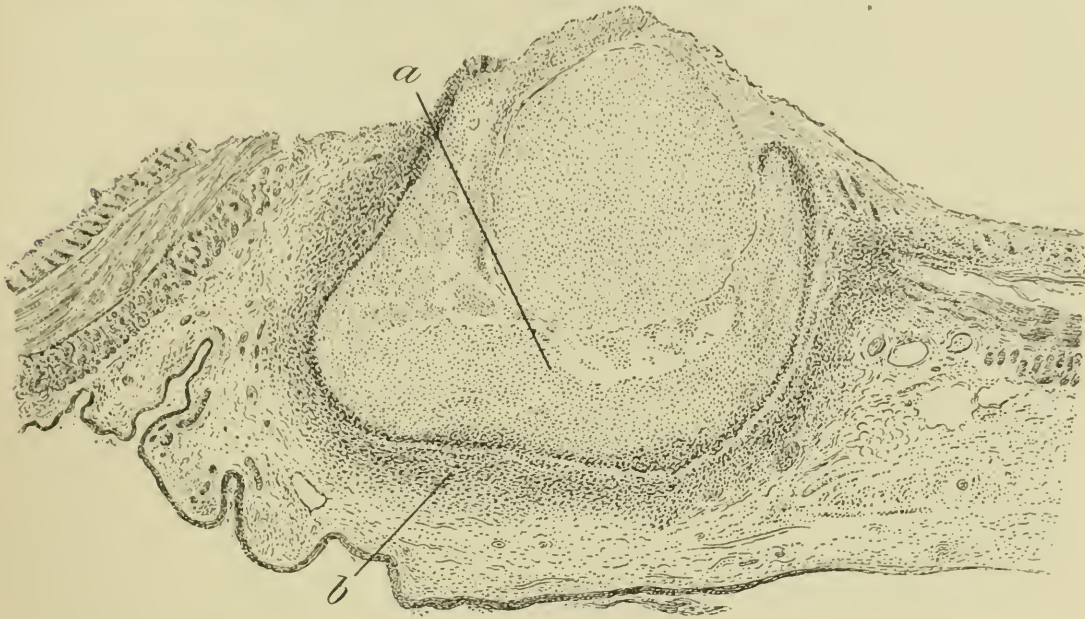


FIG. D.

FIG. D.—Section of a nodular mass implicating the several coats of the small intestine of a hog. Death on 42d day after being fed 25 cc. of a bouillon culture of *B. icteroides*. *a*, central necrosis; *b*, polynuclear leucocytes. $\times 24$.

Exp. XV.—Young pig No. 1072; weight 13½ lbs. June 27, 1899, fed 25 cc. of a 24-hour bouillon culture of *B. icteroides*, Havana. The symptoms presented by this animal were similar to those already recorded, viz., diarrhoea, loss of appetite and gradual emaciation. There was no fever. The diarrhoea was of an intermittent character. Temperature became subnormal on July 23, and remained so until the date of death, August 11, 1899. Weight after death, 7½ lbs.

Autopsy 10 hours after death. Several healed erosions on lips. Both lungs injected and dotted with ecchymoses. Abdominal cavity contains about 5 cc. of slightly turbid serum. Spleen slightly enlarged, firm, capsule thickened. Liver enlarged and of pale color. Kidney shows

thickened and cloudy cortex; pyramids pale. Stomach normal. The mucosa of the upper ileum is swollen and injected and shows circumscribed patches covered with a thin grayish exudate. At a point about 9 feet below the pylorus there are two sharply circumscribed, round, nodular masses which appear to be situated in the submucosa. They are 8 mm. in diameter and project prominently toward the peritoneal surface. The mucosa is distinctly raised by these nodules, but appears to be intact. Scattered through the ileum, singly or in groups, there are 9 other neoplastic growths of like character. Some of these involve the entire thickness of the bowel and show on the mucous surface a distinct central necrosis (see Fig. D). The central necrosis in several of these button-like masses has reached the peritoneal surface and resulted in adhesions to the omentum. A few irregular, superficial ulcers are found in the cæcum and large intestine, including the rectum.

Cultures from the blood, sterile; from the abdominal cavity and bile, the colon bacillus; from the liver, in pure culture, a few colonies of an actively motile bacillus which grows sparsely and slowly on Loeffler's blood serum, and which corresponds in its cultural characters with *B. icteroides*.

Exp. XVI.—Young pig No. 1073; weight 14 lbs. June 27, 1899, fed 25 cc. of a 24-hour bouillon culture of *B. icteroides*, Santiago. Same symptoms as in preceding experiment. No fever. July 11, animal has lost flesh; eats but little and shows weakness in hind extremities. From July 14 the temperature was subnormal till death on July 31, 1899, 34 days after feeding.

Autopsy immediately after death. Both lungs dotted with a few discrete subpleural ecchymoses; otherwise normal. Spleen enlarged, firm. Liver of light, yellowish color; fatty. No lesions in stomach. The small intestine contains a number of the sharply defined nodular masses, such as have already been described in the preceding experiment. There are also areas of diphtheritis and ulceration in the cæcum and large intestine, including the rectum. Cultures sterile.

At this time our attention was called to the negative results obtained by Wasdin and Geddings³¹ from feeding pigs with large quantities of a pure culture of *B. icteroides* in an experiment made at Delaware Breakwater. The conclusion arrived at by these observers—"that the domestic pig is incapable of infection by the *Bacillus icteroides* when introduced through the intestinal or digestive tract"—was so absolutely contradictory to the results obtained by us, that we proceeded once more

³¹ Report on the Cause of Yellow Fever. Washington, 1899.

to repeat our observations, taking care that these should be guarded by every possible precaution.

With this object in view, we procured four young pigs from the same litter. On September 7, 1899, these pigs were placed in a separate building where hogs had never been kept. Their average weight at this time was $12\frac{1}{4}$ lbs., the smallest weighing 11, and the heaviest 13 lbs. They were retained under observation until September 26, at which date their average weight was $14\frac{1}{4}$ lbs. All appeared to be in excellent condition. On this date one of the pigs was killed and autopsied, with the result that its organs and digestive tract were found to be entirely free of lesions. Cultures negative. On the same day the three remaining pigs were sent to the Soldiers' Home, Washington, D. C., where, as far as we can ascertain, hogs have never been kept. One of the pigs, weight $14\frac{1}{4}$ lbs., was placed in a separate room to serve as a control. The remaining two pigs were fed cultures of *B. icteroides*, with the following result:

Exp. XVII.—Young pig No. 1128, weight $15\frac{1}{2}$ lbs. No lesions about lips or mouth. September 27, 1899, fed 50 cc. of a 4-day bouillon culture of *B. icteroides*, original, which had recently been recovered from the blood of rabbit No. 1120. September 30, the animal has refused food and has had a slight diarrhœa. Drinks water freely. October 4, condition same; eats but little; weight 13 lbs., a loss of $2\frac{1}{2}$ lbs. in one week. The control pig on this date has gained $1\frac{1}{4}$ lbs. Was again fed 200 cc. of the same culture. October 14, animal has slowly improved in condition; has more appetite; weighs 14 lbs., a gain of 1 lb. From this date the pig recovered rapidly. November 8, weight $16\frac{3}{4}$ lbs. Animal killed. No lesion found in any of the organs or digestive tract.

Cultures from organs and mesenteric gland negative.

That this animal was made sick by feeding with *B. icteroides* was shown by the loss of appetite, transient diarrhœa, and loss of weight during the first week.

It is of interest to note, also, as pointing to infection that the serum of this pig drawn on October 28 and November 8, in dilutions of 1 to 120, caused arrest of motility and good agglutination of *B. icteroides* at the end of one hour, whereas the serum of the control pig in dilution of 1 to 20 was entirely negative.

Exp. XVIII.—Young pig No. 1130; weight $13\frac{1}{2}$ lbs. No lesions about lips or mouth. September 27, 1899, fed 50 cc. of a 4-day bouillon culture of *B. icteroides*, original, received from Roux's laboratory, and which had been cultivated on agar for the period of two years without passage through any animal. September 30, animal has been sick since the day following the feeding. Has diarrhœa and has lost flesh. October 4, diarrhœal discharges continue; weakness in hind extremities; weight

9 lbs., a loss of $4\frac{1}{2}$ lbs. in seven days. Was again fed 50 cc. of the same culture in milk, which it partially drank. Death 7 P. M., October 7, 1899, at the end of 10 days. Weight after death, $8\frac{1}{2}$ lbs.

Autopsy at noon, October 8, 1899, the body having been kept in ice-box. Two circular ulcers with indurated margins and necrotic centres on inner surface of lower lip; one measures 2.5 mm., the other 5 mm. in diameter. Inguinal glands swollen. Lungs slightly injected throughout. The abdominal cavity contains 10 cc. of clear serum. Visceral peritoneum generally injected. Mesenteric glands swollen. Spleen moderately enlarged, soft, dark in color. Liver congested, firm, markings obscure. Kidneys swollen, pale; cortex cloudy. The œsophagus is congested throughout. The fundus of the stomach is also much congested. There are, in addition, near the cardiac opening, several elevated, sharply circumscribed patches of necrosis. The mucosa of the duodenum and of the lower ileum is swollen and deeply congested. The mucosa of the cæcum shows areas of necrosis. In the large bowel this necrosis becomes diffused and general, involving the entire surface of the gut for a distance of 18 inches. Below this point there are discrete ulcers covered with bile-stained exudate. These occur also in the rectum.

Cultures from the liver and mesenteric gland, positive. Small pieces of a mesenteric gland were also placed beneath the skin of a rabbit. The latter died on the 8th day. Cultures obtained from the rabbit, as well as those from the pig's liver and mesenteric gland by direct culture, give in pure culture an actively motile bacillus which grows sparingly on blood-serum.

The control pig was killed on November 9. Weight $17\frac{1}{2}$ lbs. At autopsy no lesions whatever were found in the organs or digestive tract. Cultures negative.

We believe that the last-mentioned experiment will bear the most rigid scrutiny, and that it proves conclusively that *B. icteroides*, original, when fed to the domestic pig, will cause a fatal infection.

That an acute infection may be produced in swine not only by feeding pure cultures of *B. icteroides*, but that the disease may also be naturally acquired by these animals after exposure in infected pens is, we think, of especial importance when taken in connection with the comparison of the experimental lesions already obtained in other animals.

If we compare the lesions produced in the domestic pig infected with *B. icteroides* with those found in swine that have died of hog-cholera, it will be seen that these are practically the same, consisting of various necrotic, diphtheritic and ulcerative processes which, while affecting to a less extent the mouth, stomach and small intestine, have their chief seat, as a rule, in the large bowel.

According to Welch and Clement,³² more characteristic lesions, but less often met in experimental cases, are the so-called "buttons," viz., certain elevated, circumscribed, round or oval areas of necrotic inflammation of firm consistence, which implicate the mucous and submucous coats, and sometimes all of the coats of the bowel. The foregoing experiments will show that we have succeeded in reproducing in swine infected with *B. icteroides* all of the acute lesions of the digestive tract such as are found in hogs dead of hog-cholera. As regards the more characteristic, button-like lesions of hog-cholera, we invite attention to Exp. XV, in which these circumscribed necrotic masses involving the several coats of the bowel were found in a pig that died on the 42d day after being fed a culture of *B. icteroides*, Havana. We also observed lesions of the same character, only in an earlier stage of development, in the large intestine of pig 979, Exp. VI, and pig 994, Exp. VII.

Although we did not employ controls in our earlier experiments, we consider it of especial importance that our pigs sickened so promptly after being fed cultures of *B. icteroides*, with such symptoms as loss of appetite, choleraic diarrhœa, fever, etc., and that the bacillus recovered by us from our several autopsies always showed the same cultural characters, viz., sparseness of growth on blood-serum, limited surface growth in gelatine stab cultures, and atypical radiating colonies in gelatine plates, such as we have constantly found with *B. icteroides*, original, and *B. icteroides*, Havana.

Without entering into a description of the microscopic lesions found in swine infected with *B. icteroides*, we may state that the changes in the intestine, liver and kidneys correspond closely with those seen in experimental hog-cholera. We have not observed hyaline thrombi in the glomerular capillaries, to which Welch and Clement have called attention in swine dead of hog-cholera and in animals inoculated with the hog-cholera bacillus.

Reciprocal Immunization.

Additional evidence pointing to the close affinity of *B. icteroides* and the hog-cholera bacillus may be found,—

(a) In the protective influence in guinea-pigs of sterilized cultures of *B. icteroides* against a fatal dose of the hog-cholera bacillus.

(b) In the protective influence in guinea-pigs of sterilized cultures of the hog-cholera bacillus against a fatal dose of *B. icteroides*.

³² Remarks on Hog-cholera and Swine Plague. Proceedings of the Thirtieth Annual Convention of the United States Veterinary Medical Association and First Veterinary Congress of America, p. 206. Philadelphia, 1894.

(c) In the immunity produced in rabbits from a virulent culture of the hog-cholera bacillus by the injection of repeated doses of a living culture of *B. icteroides* of weak virulence.

(d) In the reciprocal agglutinative reactions of the sera of animals immunized with these bacilli.

(e) In the mutual reaction shown by the blood of yellow fever and hog-cholera upon *B. icteroides* and the hog-cholera bacillus.

Immunization of guinea-pigs from the hog-cholera bacillus with sterilized cultures of Bacillus icteroides.

In our first attempts to produce immunity in guinea-pigs, in which we used large doses (1 cc.) of a bouillon culture of *B. icteroides*, grown for 24 hours at 37° C., and afterwards sterilized for one hour in a water bath at 70° C., we found that our animals generally died in a much emaciated condition within about one week after receiving the first injection. We therefore substituted smaller doses (0.3 cc.), and observed that even with this quantity there was considerable loss of weight which was followed by death in some cases. It was for this reason that we postponed a second injection of the sterile culture until the guinea-pigs had begun to show a gain in weight. This usually caused the lapse of about 20 days between the several injections, as well as a considerable interval between the last immunizing dose and the injection of the virulent culture. Table III shows the results obtained.

It will be seen that of 12 pigs in which immunization was attempted, 2 died after 13 and 16 days following the first injection, and 2 within 3 and 6 days after the second injection of a sterile culture.

Of the 8 protected pigs that received 0.3 cc. of a virulent culture of the hog-cholera bacillus, one died on the 14th day, one on the 47th day and 6 recovered.

The two controls died on the 7th and 11th day respectively.

The result would appear to show that decided protection had been conferred against the hog-cholera bacillus by previous injections of sterile cultures of *B. icteroides*.

Immunization of guinea-pigs from B. icteroides with sterilized cultures of the hog-cholera bacillus.

We have also succeeded in conferring immunity upon guinea-pigs

from *B. icteroides* by previous injections of sterilized bouillon cultures of the hog-cholera bacillus. We submit the results in Table IV.

As shown in the table, 2 guinea-pigs died within 4 and 11 days after receiving the first injection of 0.3 cc. of a sterile culture of

TABLE III.

IMMUNIZATION OF GUINEA-PIGS FROM THE HOG CHOLERA BACILLUS, WITH STERILIZED BOUILLON CULTURES OF ICTEROIDES, ORIGINAL.

No.	Date of injection with sterilized culture of <i>B. icteroides</i> .	Weight in grammes.	Quantity of sterilized culture injected.	Date of injection with living culture of Hog-cholera bacillus.	Weight in grammes.	Quantity of living culture injected.	Remarks.
1	1899 May 9	415	0.3 cc.	1899	243	Died 13 days after first injection. Cultures sterile.
2	May 9	320	0.3 "				
	" 31	275	0.3 "				
	June 19	256	1.0 "	July 1	243	0.3 cc.	Died July 15, 1899.
3	May 9	355	0.3 "				
	" 31	300	0.3 "				
	June 19	296	1.0 "	July 1	293	0.3 "	Recovered.
4	May 9	455	0.3 "				Died 6 days after second injection. Cultures sterile.
	" 31	395	0.3 "	345	
5	May 9	390	0.3 "				Died 3 days after second injection. Cultures sterile.
	" 31	370	0.3 "	261	
6	May 9	400	0.3 "				
	" 31	335	0.3 "				
	June 19	347	1.0 "	July 1	348	0.3 cc.	Recovered.
7	May 9	275	0.3 "	210	Died 16 days after first injection. Not autopsied.
8	May 9	290	0.3 "				
	" 31	220	0.3 "				
	June 19	232	1.0 "	July 1	193	0.3 cc.	Recovered.
9	May 9	370	0.3 "				
	" 31	300	0.3 "				
	June 19	277	1.0 "	July 1	276	0.3 "	Died August 17, 1899.
10	May 9	335	0.3 "				
	" 31	303	0.3 "				
	June 19	321	1.0 "	July 1	338	0.3 "	Recovered.
11	May 9	340	0.3 "				
	" 31	315	0.3 "				
	June 19	316	1.0 "	July 1	327	0.3 "	Recovered.
12	May 9	385	0.3 "				
	" 31	295	0.3 "				
	June 19	278	1.0 "	July 1	312	0.3 "	Recovered.
13	(Control)	July 1	310	0.3 "	Died July 12, 1899.
14	(Control)	July 1	257	0.3 "	Died July 8, 1899.

the hog-cholera bacillus. The remaining 10 animals and 3 controls then received on June 19, 0.2 cc. of a virulent culture of *B. icteroides*, with the result that only one control died on the 13th day.

TABLE IV.

IMMUNIZATION OF GUINEA-PIGS FROM *B. ICTEROIDES*, ORIGINAL, WITH STERILIZED BOUILLON CULTURES OF THE HOG-CHOLERA BACILLUS.

No.	Date of injection with sterilized culture of Hog-cholera Bacillus.	Weight in grammes.	Quantity of sterilized culture injected.	Date of injection with living culture of <i>B. icteroides</i> .	Weight in grammes.	Quantity of living culture injected.	Remarks.
	1899			1899			
1	May 19	740	0.3 cc.	June 19	760	0.2 cc.	
	" 31	783	1.0 "	July 6	645	0.3 "	Recovered.
2	" 19	795	0.3 "	June 19	860	0.2 "	
	" 31	885	1.0 "	July 6	815	0.3 "	Recovered.
3	" 19	682	0.3 "	June 19	735	0.2 "	
	" 31	740	1.0 "	July 6	732	0.3 "	Recovered.
4	" 19	665	0.3 "	June 19	740	0.2 "	
	" 31	730	1.0 "	July 6	672	0.3 "	Recovered.
5	" 19	700	0.3 "	June 19	755	0.2 "	
	" 31	770	1.0 "	July 6	792	0.3 "	Recovered.
6	" 19	665	0.3 "	June 19	687	0.2 "	
	" 31	715	1.0 "	July 6	670	0.3 "	Recovered.
7	" 19	668	0.3 "	580	Died 11 days after first injection. Cultures sterile.
8	" 19	545	0.3 "	500	Died 4 days after first injection. Cultures sterile.
9	" 19	732	0.3 "	June 19	705	0.2 cc.	
	" 31	692	1.0 "	July 6	700	0.3 "	Recovered.
10	" 19	610	0.3 "	June 19	615	0.2 "	
	" 31	645	1.0 "	July 6	614	0.3 "	Recovered.
11	" 19	727	0.3 "	June 19	672	0.2 "	
	" 31	790	1.0 "	July 6	730	0.3 "	Recovered.
12	" 19	642	0.3 "	June 19	698	0.2 "	
	" 31	662	1.0 "	July 6	687	0.3 "	Recovered.
13	(Control)	June 19	457	0.2 "	
				July 6	375	0.3 "	Died Aug. 31, 1899.
14	(Control)	June 19	695	0.2 "	
				July 6	555	0.3 "	Died July 9, 1899.
15	(Control)	June 19	520	0.2 "	Died July 1, 1899.

On July 6, therefore, the remaining controls and the 10 protected guinea-pigs were again inoculated with 0.3 cc. of a virulent culture of *B. icteroides*. As the result of this second injection, one control

died on the 3d day and one on the 55th day following the inoculation, while the 10 immunized guinea-pigs recovered.

Immunization of rabbits from the hog-cholera bacillus with living cultures of B. icteroides.

The culture of *B. icteroides*, Havana, isolated from the cadaver of Private Patrick Smith, to whose case we have already made reference, when tested by us on guinea-pigs and rabbits, was found to be of decidedly weakened virulence as compared with the culture of this bacillus received from Roux's laboratory. We therefore selected 4 rabbits which had survived for a period of 26 days the subcutaneous inoculation of 0.3 cc. of a 24-hour culture of *B. icteroides*, Havana, and endeavored to heighten their immunity by repeated injections of

TABLE V.

IMMUNIZATION OF RABBITS FROM THE HOG-CHOLERA BACILLUS, WITH LIVING CULTURES OF ICTEROIDES, HAVANA.

No.	Date of injection with living culture of <i>B. icteroides</i> .	Weight in grammes.	Quantity of living culture of <i>B. icteroides</i> injected.	Date of injection with living culture of Hog-cholera bacillus.	Weight in grammes.	Quantity of living culture of Hog-cholera Bacillus injected.	Remarks.
1	1899 Aug. 18	2165	0.3 cc.	1899 Dec. 11	2265	0.1 cc.	Still living at end of 51 days.
	Sept. 13	0.5 "				
	Oct. 6	0.5 "				
	Oct. 26	1.0 "				
	Nov. 13	1.0 "				
2	Aug. 18	1520	0.3 "	Dec. 11	1615	0.1 cc.	Still living at end of 51 days.
	Sept. 13	0.5 "				
	Oct. 6	0.5 "				
	Oct. 26	1.0 "				
	Nov. 13	1.0 "				
3	Aug. 18	1000	0.3 "	Dec. 11	1565	0.1 cc.	Still living at end of 51 days.
	Sept. 13	0.5 "				
	Oct. 6	0.5 "				
	Oct. 26	1.0 "				
	Nov. 13	1.0 "				
4	Aug. 18	1050	0.3 "	Dec. 11	1487	0.1 cc.	Still living at end of 51 days.
	Sept. 13	0.5 "				
	Oct. 6	0.5 "				
	Oct. 26	1.0 "				
	Nov. 13	1.0 "				
5	(Control)	Dec. 11	1065	0.1 "	Died Dec. 17, 1899.
6	(Control)	Dec. 11	1060	0.1 "	Died Dec. 15, 1899.

increasing doses of the living culture. Later we injected these rabbits and 2 controls with 0.1 cc. of a virulent culture of the hog-cholera bacillus. The results are recorded in Table V.

While the controls died on the 4th and 6th day after inoculation, the immunized rabbits were alive and apparently in good condition at the end of one month and 25 days, thus demonstrating that an animal so very susceptible as the rabbit to *B. icteroides* and the hog-cholera bacillus may be rendered immune from a fatal dose of the hog-cholera bacillus by repeated injections of a living culture of *B. icteroides* of weak virulence.

Reciprocal Agglutinative Reactions.

Reciprocal agglutinative reaction of the sera of animals immunized from B. icteroides and the hog-cholera bacillus.

Our first observation was made with the serum of a dog which had been partly immunized by the intravenous injection of increasing doses of *B. icteroides*. Serum obtained from this dog at the end of three months, tested in a dilution of 1 to 5,000, brought about prompt arrest of motility of the hog-cholera bacillus followed by agglutination at the end of one hour. The death of this dog prevented further tests of the serum.

A second observation made with a specimen of "anti-amaryllic" serum received from South America, showed that while *B. icteroides* was agglutinated in a dilution of 1 to 120,000, it required a dilution of 1 to 30,000 to bring about a like agglutination of the hog-cholera bacillus at the end of one hour.

A third observation was made with the blood-serum of a dog which had been injected with gradually increasing doses of the bacillus of hog-cholera, during a period of three months. With this serum, the hog-cholera bacillus was agglutinated in a dilution of 1 to 2,000, and the Sanarelli bacillus in a dilution of 1 to 600, at the end of one hour.

These several sera, in a dilution of 1 to 20, were entirely negative in their reaction toward the typhoid bacillus and *Bacillus coli communis*.

Agglutinative reaction of the blood of yellow fever upon B. icteroides and the hog-cholera bacillus.

Sanarelli³³ has observed that the serum of the blood of yellow-fever cadavers produces in cultures *in vitro* of *B. icteroides* the phenomenon of agglutination, but that the intensity of this reaction is very variable. The serum obtained in one case on the 17th day of convalescence from yellow fever produced very slight agglutination.

Archinard and Woodson,³⁴ using the dried blood of yellow-fever patients, in the estimated dilution of 1 to 10 to 1 to 40, obtained in over 70 per cent of a series of 50 cases examined cessation of motion and agglutination, "the reaction being as characteristic as in typhoid fever cases." Accordingly they urge the practical value of the serum diagnosis of yellow fever. In a later paper,³⁵ these observers report that the reaction is present in over 80 per cent of cases of yellow fever or of recent convalescents.

Wasdin and Geddings³⁶ failed to confirm the observations of Archinard and Woodson. As the outcome of their experience with the test, using the blood of yellow fever patients and the blood of animals sick or dead of inoculation with *B. icteroides*, they state: "The results were most varying and bewildering and convince us that whatever may be the value of the reaction as a diagnostic point in enteric fever, it has little or none in yellow fever."

We have, through the kindness of Acting Assistant Surgeon A. Agramonte, U. S. Army, on duty in Havana, Cuba, received specimens of blood from a number of cases of yellow fever. These we have subjected to careful tests, using the method suggested by Wyatt Johnston which we have uniformly found satisfactory for testing the agglutinative reaction of the blood of typhoid fever. The dilution used by us was approximately 1 to 30. In addition to making duplicate tests of this blood with *B. icteroides* original, we have also tested its action on the hog-cholera bacillus and on *Bacillus A* (Archinard

³³ L'immunité et la sérothérapie contre la fièvre jaune. *Annales de l'Institut Pasteur*, 1897, xi, p. 753.

³⁴ The Serum Diagnosis of Yellow Fever. *New Orleans Medical and Surgical Journal*, 1898, 1, p. 455.

³⁵ Bacteriological study in the etiology of yellow fever. *New York Medical Journal*, 1899, lxi, p. 109.

³⁶ *Op. cit.*, p. 78.

and Woodson),³⁷ since the latter bacillus responded to agglutination equally with the Sanarelli bacillus in the hands of these observers. We present the results in Table VI.

TABLE VI.
AGGLUTINATION TESTS WITH DRIED BLOOD FROM CASES OF YELLOW FEVER.—
ESTIMATED DILUTION 1 TO 30.—TIME 4 HOURS.

No.	Case.	Day of illness.	<i>B. icteroides</i> , (Original).	Hog-cholera bacillus.	Bacillus "A" (Archinard & Woodson).
1	A. J. Bathon. (Doubtful case).....	8th	Negative.	Negative.	Negative.
2	Robert Stewart. (Undisputed case).....	9th	"	"	"
"	" " "	12th	"	"	"
3	Wm. Kehrler. (Fatal case).....	4th	"	"	"
4	Tom Buchanan. (Typical case).....	5th	"	"	"
5	Mike Deveney. (Typical case).....	5th	"	"	"
6	G. P. Thomas. (Typical case).....	13th	"	"	"
7	J. G. Thatcher. (Typical case).....	9th	"	"	"
8	George Woods. (Typical case).....	7th	"	"	"
9	Lant Shears. (Typical case).....	4th	"	"	"
10	Wm. Demuth. (Typical case).....	4th	Positive at end of 1½ hours.	Positive at end of 1½ hours.	Partial ar- rest of motil- ity with some agglutina- tion.
11	B. Dadd. (Typical case).....	5th	Negative.	Negative.	Negative.
12	Wm. Shaw. (Typical case).....	12th	"	"	"
13	Fred Worrell. (Typical case).....	Convalescent	"	"	"
14	Wm. J. Mooney. (Typical case—fatal)...	2d, 14 days before death.	"	Not suffi- cient blood for a fair test.	"
15	Jas. A. Hays. (Typical case—fatal)...	1st day af- ter admis- sion, 2 days before death.	"	Negative.	"

³⁷ This actively motile bacillus (A) which was isolated by Archinard and Woodson in 32 of 39 autopsies of yellow-fever cases, (4 times in 5 cases from the blood taken at the elbow), differs from *B. icteroides* (Sanarelli) in its more rapid growth and tendency to spread in gelatine plates. The colonies are large, and of irregular outline. It does not coagulate milk nor bring about opalescence in this medium; it ferments glucose, but not lactose or saccharose; grows freely on blood serum and gives only a slight indol reaction. It belongs to the hog-cholera group and corresponds closely in its cultural characters with *B. enteritidis* (Gärtner). It is not a form of *Bacillus coli communis* as stated by Wasdin and Geddings (Report on the Cause of Yellow Fever, p. 16).

TABLE VI—*Continued.*

No.	Case.	Day of illness.	B. icteroides, (Original).	Hog-cholera bacillus.	Bacillus "A." (Archinard & Woodson).
16	Luis Colome. (Typical case—recovered)	6th	Negative.	Negative.	Negative.
17	J. J. Dougherty. (Typical case)	7th	"	"	"
18	Burton Fowler. (Typical case)	8th	"	"	"
"	" "	12th	"	"	"
"	" "	15th	"	"	"
19	Anthony Wiedner. (Typical case)	5th	"	"	"
"	" "	12th	"	"	"
20	Timothy Healy. (Typical case)	5th	"	"	"
"	" "	9th	"	"	"
21	Stephen Scanlan	6th	"	"	"
22	Wm. Harper	Convalescent	"	"	"
23	Jess Hilton	9th	"	"	"
24	Chas. Mitchell	7th	"	"	"
25	Max Thompson	9th	"	"	"
26	Dan. Coleman	9th	"	"	"
27	Chas. Rodgers	8th	"	"	"
"	" "	13th	"	"	"
28	Sigismund Fichman	6th	"	"	"
"	" "	9th	"	"	"
"	" "	12th	"	"	"
29	Feodora Fernandez	8th	"	"	"

It will be seen that only one of 29 (3.4 per cent) samples of yellow fever blood, taken on various days of the disease, or during convalescence, exhibited any agglutinative reaction towards *B. icteroides*; and, further, that this specimen of blood was also positive in its agglutinative effect upon the hog-cholera bacillus.

Our observations, therefore, while agreeing with those of Wasdin and Geddings, do not confirm the results obtained by Archinard and Woodson as to the agglutinative reaction of the blood of yellow fever upon *B. icteroides*.

Agglutinative reaction of the blood of hog-cholera upon the hog-cholera bacillus and B. icteroides.

We also submit in Table VII the results of tests made with fluid serum, dried blood and dried serum obtained, through the kind assistance of Dr. E. A. de Schweinitz, U. S. Department of Agriculture,

from hogs affected with hog-cholera. The material was obtained immediately after death from animals that were killed and upon post-mortem examination pronounced to be cases of hog-cholera by the veterinarian.

TABLE VII.

AGGLUTINATION TESTS WITH FLUID SERUM, DRIED BLOOD AND DRIED SERUM
FROM HOGS SUPPOSED TO BE SUFFERING WITH HOG-CHOLERA.—
TIME 2 TO 4 HOURS.

No.	Material used.					Hog-cholera bacillus No. 1.	<i>B. icteroides</i> , (Original).
1	Fluid Serum.	Dilution 1 to 30.				Negative.	Negative.
2	"	"	"	"	"	Positive.	Positive.
3	"	"	"	"	"	"	"
4	"	"	"	"	"	Motility arrested. No agglutination.	Motility arrested. No agglutination.
5	Dried Blood.	Estimated dilution 1 to 30.				Negative.	Negative.
6	"	"	"	"	"	"	"
7	"	"	"	"	"	"	"
8	"	"	"	"	"	Positive.	Positive.
9	"	"	"	"	"	Negative.	Negative.
10	"	"	"	"	"	Motility arrested. No agglutination.	Motility arrested. No agglutination.
11	"	"	"	"	"	Negative.	Negative.
12	"	"	"	"	"	"	"
13	"	"	"	"	"	Positive.	Positive.
14	"	"	"	"	"	"	"
15	"	"	"	"	"	Negative.	Negative.
16	"	"	"	"	"	"	"
17	"	"	"	"	"	Impairment of motility with slight agglutination.	Impairment of motility with slight agglutination.
18	"	"	"	"	"	Negative.	Negative.
19	"	"	"	"	"	"	"
20	"	"	"	"	"	Impairment of motility with slight agglutination.	Impairment of motility with slight agglutination.
21	"	"	"	"	"	Negative.	Negative.
22	"	"	"	"	"	"	"
23	"	"	"	"	"	"	"
24	"	"	"	"	"	"	"
25	"	"	"	"	"	"	"
26	"	"	"	"	"	"	"
27	Dried Serum.	Estimated dilution 1 to 30.				"	"
28	"	"	"	"	"	"	"
29	"	"	"	"	"	Positive.	Positive.
30	"	"	"	"	"	Negative.	Negative.
31	"	"	"	"	"	"	"
32	"	"	"	"	"	"	"

Thus, of 32 samples of serum or dried blood from cases pronounced to be hog-cholera, 6, or 18.75 per cent, gave a positive reaction, and 4, or 12 per cent, a partial reaction with both the hog-cholera bacillus and *B. icteroides*.

At what period of the disease these specimens of blood were taken, we have no positive means of determining. We think, however, that the results obtained with *B. icteroides* when taken in connection with the negative reaction shown by the blood of yellow fever are very suggestive.

CONCLUSIONS.

1. *Bacillus X* (Sternberg) belongs to the colon group.
2. *Bacillus icteroides* (Sanarelli) is a member of the hog-cholera group.
3. The various channels of infection, the duration of the disease and the gross and microscopical lesions in mice, guinea-pigs and rabbits are the same for *Bacillus icteroides* and the hog-cholera bacillus.
4. The clinical symptoms and the lesions observed in dogs inoculated intravenously with *Bacillus icteroides*, are reproduced in these animals by infection with the hog-cholera bacillus.
5. *Bacillus icteroides* when fed to the domestic pig causes fatal infection, accompanied by diphtheritic, necrotic and ulcerative lesions in the digestive tract, such as are seen in hogs when infected with the hog-cholera bacillus.
6. This disease may be acquired by exposing swine in pens already infected with *Bacillus icteroides*, or by feeding them with the viscera of infected pigs.
7. Guinea-pigs may be immunized with sterilized cultures of *Bacillus icteroides* from a fatal dose of the hog-cholera bacillus and *vice versa*.
8. Rabbits may be rendered immune by gradually increasing doses of a living culture of *Bacillus icteroides* of weak virulence from a fatal dose of a virulent culture of the hog-cholera bacillus.
9. The sera of animals immunized with *Bacillus icteroides* and with the hog-cholera bacillus, respectively, show a marked reciprocal agglutinative reaction.

10. While the blood of yellow fever practically does not exercise an agglutinative reaction upon *Bacillus icteroides*, the blood of hog-cholera agglutinates this bacillus in a much more marked degree, thus pointing, we think, to the closer etiological relationship of this bacillus to hog-cholera than to yellow fever.³⁸

DESCRIPTION OF PLATE XIX.

Fig. 1. Photomicrograph showing focal necrosis in the liver of a guinea-pig. Death on 6th day after subcutaneous inoculation with *B. icteroides*. See pp. 240-242. $\times 100$.

Fig. 2. Photomicrograph of section of human liver showing focal necrosis invaded by leucocytes; no fatty degeneration. From case of Patrick Smith, 8th infantry. Death on 9th day of illness. *B. icteroides* isolated from blood 4 days before death. Typhoid bacillus isolated from spleen at autopsy. See p. 248. $\times 100$.

Fig. 3. Photomicrograph of section of human liver in yellow fever, showing fatty degeneration. $\times 100$.

³⁸ In a preliminary note on "The Etiology of Yellow Fever," by Reed, Carroll, Agramonte and Lazear (*Philadelphia Medical Journal*, Oct. 27, 1900), the authors state that they failed to find *B. icteroides* either in the blood during life of 21 patients in various stages of yellow fever or in cultures from the blood and organs at 11 autopsies of yellow-fever patients.

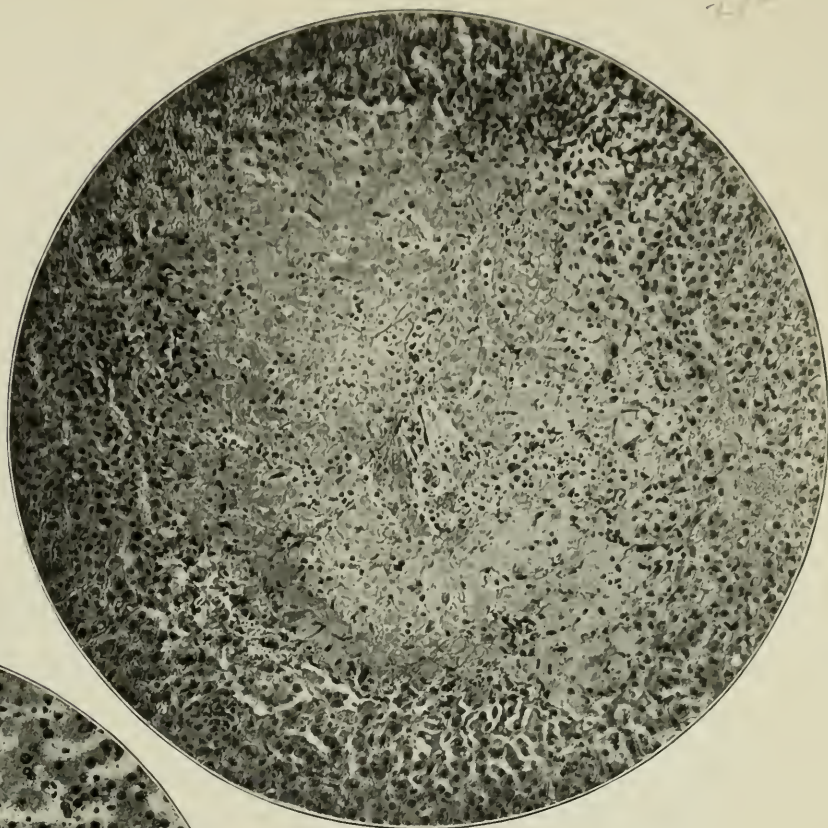


FIG. 1.

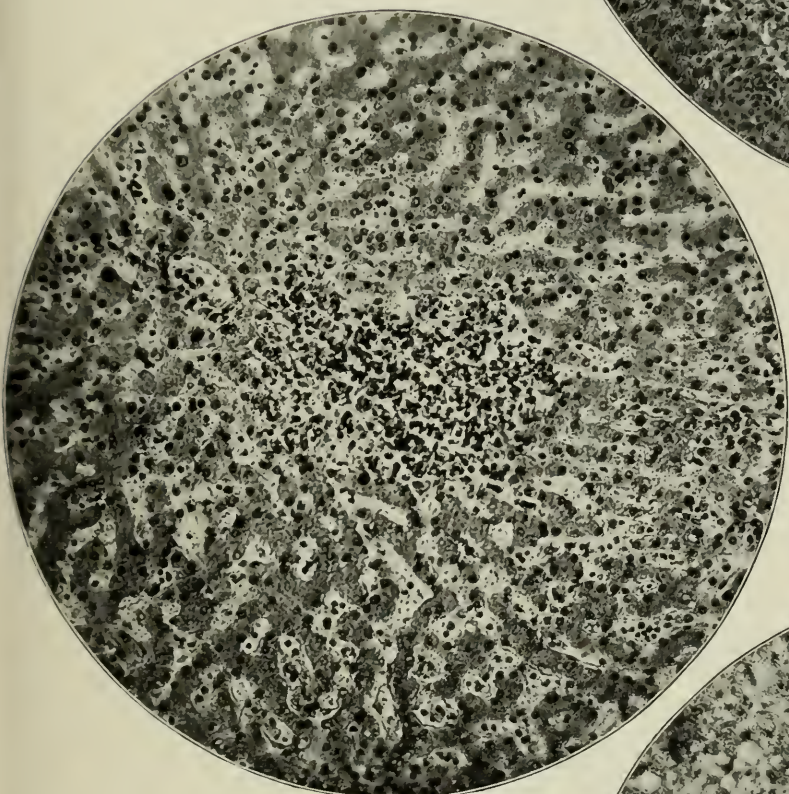


FIG. 2.

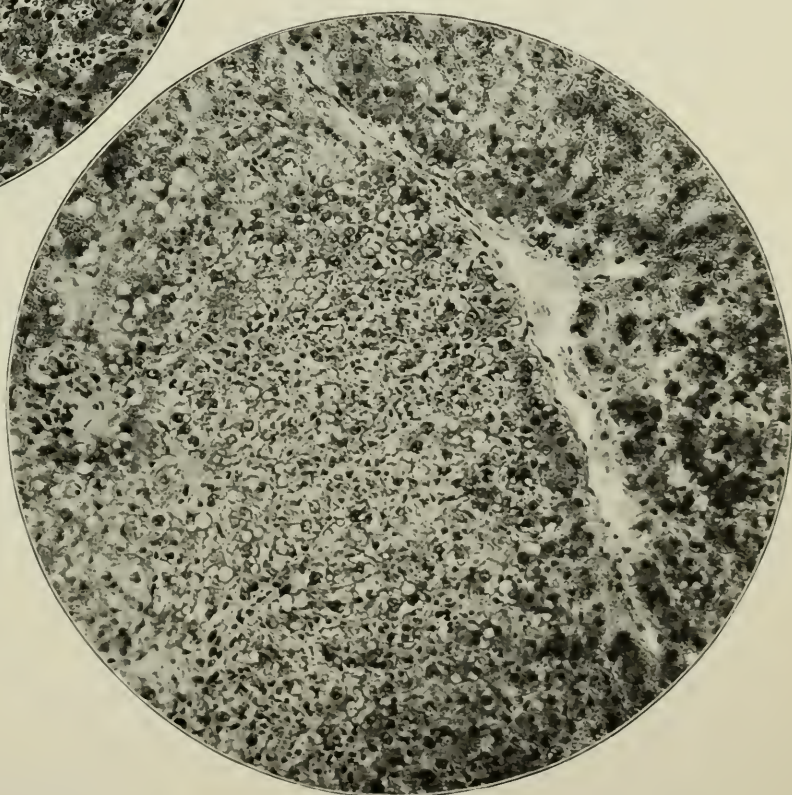


FIG. 3.

SOME OBSERVATIONS UPON THE BACTERIAL SELF-PURIFICATION OF STREAMS.¹

BY EDWIN OAKES JORDAN, PH. D.

(*From the Bacteriological Laboratory of the University of Chicago.*)

PLATE XX.

The subject of the self-purification of streams divides itself naturally into two parts: that relating to the disappearance or oxidation of certain chemical constituents of sewage, and that relating to the disappearance of the sewage bacteria. It is of the latter only that I purpose to treat in this article. Both phenomena may properly be classed as processes of self-purification. They may not, however, have anything else in common; for it is certainly true that the process of nitrification and the processes leading to the disappearance of dangerous or suspicious bacteria do not always run a strictly parallel course either in sewage tanks or in polluted rivers. While we must not fail to recognize the possibility that a nuisance may be created through the decomposition of large quantities of organic matter in a flowing stream, it is with the fate of the bacteria rather than with that of the lifeless organic matter that sanitary investigations are most directly concerned, and so long as bacteriologists are unable to correlate more exactly than at present the disappearance of disease germs with the successive changes in the decomposition and oxidation of organic matter, the fate of disease germs introduced into a stream can be determined chiefly by inference from the known fate of the other sewage bacteria. For these reasons the number of bacteria found at various points along the course of a polluted stream possesses comparatively great significance.

¹ The observations recorded in this paper were made during a study of the chemical and bacterial condition of the Illinois River and its tributaries, undertaken in behalf of the Sanitary District of Chicago. I am indebted to Dr. Arthur R. Reynolds, Director of the Streams Examination, for permission to publish the facts at this time.

One of the first and most comprehensive studies bearing upon the bacterial self-purification of streams was made by G. Frank (1888), and was carried out upon the river Spree above and below Berlin. The general conclusions drawn from Frank's study are that the river Spree, at its entrance to the city, contains usually under 10,000 bacteria per cubic centimetre, and that this number is rapidly augmented on passing through the city until numbers rising into the hundreds of thousands and occasionally into the millions are found. Below the city the Spree expands into a lake, and the numbers of bacteria shown by the ordinary plate-count are here much lower. The following table of averages illustrates the degree of bacterial purification observed by Frank:

SPREE AT BERLIN. AVERAGE NUMBER OF COLONIES FROM FRANK'S TABLES.

	No. colonies per cubic centimetre.		No. colonies per cubic centimetre.
Oberbaumbrücke	9400	Moabiter Brücke	51500
Janowitzbrücke	13400	Spandau	340000
Friedrichsbrücke	26700	Pichelsdorf	201300
Ebertsbrücke	38000	Gatow	132200
Marschallbrücke....	31600	Cladow	178000
Moltkebrücke	69300	Sacrow	9200

At Pichelsdorf the Spree widens out into the broad lake known as Havelsee, and at Sacrow, some 13 kilometres below Pichelsdorf, near the lower end of the lake, the number of bacteria is shown to have suffered a remarkable diminution. There are a few features of Frank's work that are difficult to understand without a full knowledge of the local situation, such, for instance, as the numbers obtained at Gatow (9000), Cladow (8400) and Sacrow (11,100) on October 20, 1886, and at Pichelsdorf (6300) and Cladow² (23,900) on December 1, 1886. There can be no doubt that the value of Frank's work is somewhat impaired by his failure to secure cross-sections of the stream at the points chosen for collection and by the delay in plating caused by the transportation of the samples to the laboratory. The large number of analyses that were made (22 from each station), however, and the fact that all seasons of the year are represented, render the assumption of a great bacterial decrease between Pichelsdorf and Sacrow most plausible. More recently Dirksen and Spitta (1899) have independently arrived at virtually the same conclusion on the basis of observations made ten years later, although their methods, like those of Frank, are not above reproach. The controversy between these latter authors and Frank (1899) in respect

² About eight kilometres below Pichelsdorf.

to the interpretation of the pollution occurring during the passage of the Spree through Berlin does not alter the fact of a subsequent purification. Dirksen and Spitta, indeed, state distinctly that their results confirm the existence of a bacterial purification in the basin between Pichelsdorf and Sacrow.

The work of Girard and Bordas (1893) upon the purification of the Seine between Paris and Rouen fails to illuminate the question of self-purification very much, both on account of the lack of definite statements as to the methods employed, and because the results are not recorded in categorical form, but are simply portrayed in curves. Taking their work as it stands, a considerable but not striking degree of purification is indicated.

A comprehensive investigation extending over several years has been conducted by W. Prausnitz and others upon the river Isar, and the results have been recently summed up by Goldschmidt and Prausnitz and their collaborators (1898). The number of bacteria in the Isar is greatly increased through the addition of the sewage of Munich. At Unter-Föhring, just below Munich, the mixing of sewage and river water is presumed, by these investigators, to be complete, although unfortunately no cross-sections were obtained at this point. The following table gives the relative numbers of bacteria found at Unter-Föhring and stations below, the number at Unter-Föhring (or at Ismaning, 6 km. below Unter-Föhring) being taken as 100.

ISAR BELOW MUNICH (PRAUSNITZ AND OTHERS).

	Distance from Unter-Föhring in kilometres.	1890.	1892-93.	1889.	1890.	1893 (Dec.)	1895-96.
Unter-Föhring..	0	100	100
Ismaning	6	94.3	..	100	100	100	100
Freising	26	48.1	47.1	52.9	51.0	47.4	55.6
Landshut.....	65	29.0	23.2	..	30.7	13.1	20.5

The flow from Föhring to Freising requires about eight hours in the winter months and nearly twice as long during low-water periods. The authors conclude that in the course of about 20-26 kilometres (eight hours' flow) fifty per cent of the germs perish. This conclusion must be accepted, however, with reserve, since the authors have failed to obtain accurate data concerning mixing and dilution, two factors which by our own observations and those of Kruse (1899) are shown to possess great significance.

The work of Schlatter (1890) upon the contamination and subsequent purification of the Limmat in its passage through Zürich, although also

open in some degree to the foregoing criticisms, indicates that a considerable purification takes place in the Limmat in the course of 10 kilometres. A velocity of about 0.5 metre per second (at low water) is apparently accompanied by a notable bacterial diminution, but a velocity of 1.25-1.5 m. per second (at high water) seems to be associated with a bacterial increase. The conditions prevailing in the Limmat have been studied more recently also by Thomann (1900), whose observations are quite extended and whose results confirm in the main those of Schlatter, although they do not include observations upon high-water conditions.

Very comprehensive investigations into the bacterial changes occurring in the Rhine between Cologne and Düsseldorf have been made by Stutzer and Knublauch (1894), and by Kruse (1899) and Lossen (1899) with, however, almost diametrically opposite results. Stutzer and Knublauch's conclusions are based upon averages of plate counts obtained at various times between April and November upon samples transported to the laboratory under the usual precautions. The relative numbers are shown in the following table, the number found at Marienburg just above Cologne being considered as 100.

RHINE BELOW COLOGNE (STUTZER AND KNUBLAUCH).

	Left bank.	Midstream.	Right bank.
Marienburg (above Cologne).....	100	100	100
22 kms. below Marienburg (Langel) ...	354	214	283
27 kms. below Langel (Vollmerswerth).....	122	125	143

The effect of the contribution of the sewage and refuse from Cologne, as well as the effect caused by the entrance of the small and highly polluted tributary known as the Wupper, are shown by the figures at Langel, while at Vollmerswerth, 27 kilometres below Langel, a remarkable diminution seems to have taken place. It must be noticed, however, that sources of error incident to the methods employed by Stutzer and Knublauch may have materially influenced their results.

The work of Kruse and Lossen is of a more rigorous character. An attempt was made by these investigators to follow as far as practicable a definite body of water from Marienburg to Vollmerswerth, and with this purpose in view, hourly samples, seven in number, were collected at Marienburg and plated immediately. Four and one-half hours later a second series of platings was begun at Hitdorf (opposite Langel), 22 kilometres below Marienburg, at which time it was calculated that the body of water examined at Marienburg would begin to pass the latter point. The third series was collected at Vollmerswerth, 27 kilometres below Hitdorf, beginning at four o'clock in the evening and continuing at

hourly intervals until one o'clock in the morning. The results of this most interesting series of observations are shown in the following averages:

RHINE BELOW COLOGNE (KRUSE AND LOSSEN).

	No. of hourly analyses.	Left bank.	Midstream.	Right bank.
Marienburg (above Cologne) . .	7	8700	8900	8400
22 kms. below Marienburg (Hitdorf, opposite Langel) .	8	33450	12850	17900
27 kms. below Hitdorf (Voll- merswerth)	9	19400	17300	17400

Upon the basis of these figures, taken together with the similar issue of other examinations, the authors conclude that the upshot of their work is not favorable to the assumption of a bacterial self-purification in this part of the Rhine during a flow of 27 kilometres. No statements are made by the authors as to the measurement of the rate of flow, but it appears from the context that the rate was about 27 kilometres in $5\frac{1}{2}$ to 6 hours, or about 3 miles an hour. Experiments of a similar character made upon a longer stretch of the Rhine in a different region and at another season gave a somewhat different result as is shown in the table:

RHINE ABOVE COBLENTZ (KRUSE AND LOSSEN).

	No. of colonies per cc.
Niederwalluf (0 km.)	9500
Rüdesheim (20 km.)	7500
Assmannshausen (25 km.)	6100
St. Goar (48 km.)	5450
Oberlahnstein (68 km.)	5200

In the opinion of the authors this slow but steady decrease in the number of bacteria is not to be attributed to dilution, and they are inclined to explain this divergence from the results obtained below Cologne by a difference in the relative strength of the sunlight at the two periods, the observations recorded for the river below Cologne being made on the 10th of November, while those for the stretch above Coblenz were made during bright sunny days in September. Since the observations of Stutzer and Knublauch were made during different seasons of the year, it is possible that this factor may also help to explain the lack of agreement between their results and those of Kruse.

Among other of the more important observations that need be mentioned are those of Heider (1893) upon the Danube, Blasius and Beckurts (1895) upon the Oker, and Dräer (1895) upon the Pregel.

The outcome of all these observations can hardly be regarded as thoroughly conclusive. The methods that have been employed are far from being uniform and are frequently without precision; observations are too few or cover too limited periods of time; different parts of a stream are studied at different seasons of the year, thereby introducing grave error; important influences such as mixing and dilution are often entirely ignored. These are a few of the objections that in one form or another can be urged against many of the conclusions regarding the bacterial self-purification of streams and it must be admitted that they are quite sufficient to preclude general deductions.

The trend of the work that has been done is, however, unmistakable. A lessening of the bacterial content, whether due to dilution, to sedimentation or to the action of sunlight, stands out in some cases too clearly to admit of question. A second fact is equally salient. Great differences in the degree of this apparent purification plainly exist; these differences are dependent upon a variety of factors such as the amount of initial pollution, the velocity of flow, the season of year, etc. In the case of each stream these conditions are different and the factors necessarily possess different values. To determine, therefore, the extent to which bacterial purification occurs in a given stream, and to what it is due, is a matter for detailed special observation; inferences made on the basis of experience obtained elsewhere under a different set of conditions can have little weight in the present state of our knowledge.

An opportunity for studying the phenomena of bacterial purification under peculiarly favorable conditions has been offered me during the course of investigations upon the Illinois River, undertaken in 1899-1900 at the request of Dr. Arthur R. Reynolds, Director of the Streams Examination for the Sanitary District of Chicago. A detailed report, covering the routine chemical and bacterial analyses throughout the period under consideration is in preparation and will shortly be issued. I am enabled here, through the courtesy of Dr. Reynolds, to summarize a portion of these results, together with the record of some special studies.

The local conditions leading to this inquiry may first be briefly described. A portion of the sewage of the city of Chicago has for many

years been allowed to flow directly into Lake Michigan, which has served at once as the recipient of the city refuse and the source of the city water supply. The majority of the city sewers, however, have debouched into Chicago River, a small stream flowing normally towards the lake. The growth of the city of Chicago in the last few decades has led to so great an increase in the amount of sewage poured into the Chicago River that this stream has become practically an open sewer. As long ago as 1865 the uneasiness that was naturally felt regarding the effect of such a condition upon the public health found expression in the use of a pumping station situated at Bridgeport at the junction of the Illinois and Michigan Canal with the South Branch of the Chicago River (see map, Plate XX). Since that year the river water has been more or less regularly pumped into the canal, with the effect under ordinary conditions of reversing the flow of the river and causing a sluggish current to set away from the lake. This has served the purpose of keeping much sewage out of the drinking water. Fluctuations in the level of the lake and river, however, have not infrequently proved too much for the pumps at Bridgeport to cope with and at times of a sudden rise in the river or lowering in the lake-level crude sewage has been borne far out into the lake. The natural result of this frequent and serious pollution of the water-supply has been that Chicago has suffered severely and continuously from typhoid fever, and the municipal authorities soon came to a realization of the fact that some measure of relief was imperative. The measure selected was the construction of a drainage channel connecting the Chicago River with the Desplaines River, and thence conducting by gravity flow the sewage of the city—which must, according to legal enactment, be diluted twenty times with the water of Lake Michigan—into the valley of the Desplaines and Illinois. This channel, after some ten years of labor and the expenditure of about \$35,000,000, was finally opened in January, 1900.

Since alarm has been expressed by some of the inhabitants of the Illinois and Mississippi Valleys, and particularly by the city of St. Louis, which derives its water-supply from the Mississippi River about thirty miles below the mouth of the Illinois, it seemed important to the trustees who controlled the construction of the canal to undertake a comprehensive study of the character of the Illinois River and its tributaries with a view to determining the effect of opening the canal. To this end weekly samples were collected at chosen points during the period between May 1, 1899, and January 1, 1900. The amount of sewage passing into the Illinois River by way of the Illinois and Michigan Canal throughout this period is estimated to be as high as 85-90 per cent of the total sewage of Chicago.

The reasons governing the selection of the particular points of collection may first be stated, with frequent references to the accompanying map (Plate XX).

1. *Bridgeport*.—The sample taken here represents the quality of the water pumped into the Illinois and Michigan Canal. Besides much house sewage, a large amount of manufacturing waste, including the discharges from several gas houses, is poured into the river near this point. The general character of the water is shown by the quantity of the more significant chemical constituents.

AVERAGE OF 27 DETERMINATIONS, APRIL 27, 1899, TO JAN. 1, 1900.

Parts per million.		Parts per million.	
Chlorine	119.2	Oxygen consumed.....	30.3
Free ammonia.....	15.9	Total residue on evaporation.	562.0
Albuminoid ammonia...	2.64	Suspended residue	80.9

2. *Lockport*.—The water pumped into the Illinois and Michigan Canal flows sluggishly³ some 29 miles from Bridgeport to Lockport, where the second sample was taken. Practically no dilution and no additional pollution occur on the way. The chemical impurities change but little between Bridgeport and this point.

AVERAGE OF 32 DETERMINATIONS, MAY 5, 1899, TO JAN. 1, 1900.

Parts per million.		Parts per million.	
Chlorine	117.4	Oxygen consumed.....	32.3
Free ammonia.....	15.7	Total residue on evaporation.	562.0
Albuminoid ammonia.....	2.42	Suspended residue	76.9

3. *Desplaines River*.—Below Lockport the Illinois and Michigan Canal unites with the Desplaines River, a small stream of variable volume. At this point, therefore, the sewage in the canal receives its first dilution. During the greater part of the year this dilution is very slight; but in flood season the flow in the Desplaines may amount to 800,000 cubic feet per minute. The Desplaines River itself receives some sewage from the suburban towns along its banks.

4. *Desplaines River at Joliet (above town)*.—About four miles below the union of the canal with the river the latter flows through the city of Joliet. At this point, where the canal and river are one, a sample was taken above the main part of the town. The river and canal separate a short distance below.

5. *Desplaines River at Joliet (below town)*.—A considerable proportion

³ About one-half to nine-tenths of a mile an hour.

of the sewage of Joliet enters the Desplaines River between stations (4) and (5). Joliet is a manufacturing town with a population of 23,265, according to the U. S. Census of 1890. Much manufacturing waste, as well as the major part of the house sewage, enters the river. Owing to changes made in the river bed at this point during the construction of dams and retaining walls for the Sanitary District, this collecting station, which was originally selected for the purpose of showing the extent of pollution introduced between (4) and (5), had to be temporarily abandoned.

6. *Kankakee River at Wilmington.*—The first really considerable dilution of the Chicago sewage comes from the union of the sewage-laden Desplaines with the Kankakee. The mean discharge from the Kankakee for the entire year is estimated at about 300,000 cubic feet per minute. The sample taken at Wilmington (population, 1890, 1576) gives a fair idea of the composition of this water. The organic matter found in the Kankakee is largely of vegetable origin and is derived from the extensive marshes drained by this stream. Some house sewage also enters the river at Kankakee (population, 1890, 9025), about 20 miles above Wilmington.

7. *Illinois River at Morris.*—This is the first collecting station on the Illinois River proper, and is nine and one-half miles below the junction of the Kankakee and Desplaines. The river at this point is practically a more or less complete mixture of Chicago sewage and Kankakee River water. In a dry season like that of 1899, the proportion of Kankakee water may be very small. The average volume of the Kankakee is four or five times that of the Desplaines, but owing to the fact that the mixing at Morris is incomplete, the chlorines given in the table on p. 287 do not show this clearly. A better idea of the conditions at Morris is obtained from some special determinations (p. 306-308).

8. *Fox River at Ottawa.*—The Fox River constitutes another great diluting factor. The area drained by the Fox (2697 square miles) is about one-half that drained by the Kankakee (5146 square miles), but the volume is relatively large.

9. *Illinois River at Ottawa.*—The collection at this point was made above the entrance of the Fox River and above the point where the town sewage enters, and shows the change resulting from a twenty-four mile flow without much dilution and without material addition of impurities.

10. *Big Vermilion River at La Salle.*—This is another important tributary draining an area of 1413 square miles, and receiving sewage principally from the towns of Streator (population, 1890, 11,414) and Pontiac (population, 1890, 2784).

11. *Illinois River at La Salle.*—The sample at La Salle (population 1890, 9855) was collected at a point below the entrance of the major part of the town drainage and below the mouth of the canal (see map).

12. *Illinois and Michigan Canal at La Salle.*—At La Salle the Canal finally discharges into the river (see map). Between Joliet and La Salle considerable dilution takes place, more, in fact, than in the river water (cf. chlorines).

13. *Illinois River at Henry.*—Between La Salle (11) and Henry (population, 1512) there is little dilution and practically no additional pollution.

14. *Illinois River at Averyville.*—The collecting station is at the "Narrows," about three miles above the city of Peoria, and the results obtained here show the degree of bacterial purity attained after the flow of 130 miles from Lockport.

15. *Illinois River at Wesley City.*—The city of Peoria (population, 1900, 56,100) contributes a large amount of organic refuse to the river between (14) and (15). Not only does the main part of the house sewage enter, but there is also a great addition of manufacturing waste, of distillery slop, of discharges from glucose factories, and of the sweepings of extensive stockyards. The Wesley City collection is about four miles below this outpour of pollution. The amount of the Peoria pollution varies greatly at different seasons of the year and at different hours of the day, a fact that aids in explaining the great irregularities and fluctuations in the number of bacteria observed at this point.

16. *Illinois River below Pekin.*—More house sewage and some distillery slop enter the river between (15) and (16). (Population of Pekin, 1890, 6347.)

17. *Illinois River at Havana.*—(Population, 1890, 2525.) The collection here was made above the town and above the mouth of Spoon River (drainage area, 1905 square miles).

18. *Sangamon River at Chandlerville.*—Drainage area, 5592 square miles. The sewage of Springfield (population, 1890, 24,963) enters a tributary of this stream about 45 miles above Chandlerville.

19-20. *Illinois River at Beardstown and Kampsville.*—Little organic impurity is added to the river below the mouth of the Sangamon. There is no sewered town on the Illinois between Beardstown and Grafton.

21. *Illinois River at Grafton.*—This is the last collecting station on the river before the union of the latter with the Mississippi.

22. *Mississippi River at Grafton.*—The collection here was made above the mouth of the Illinois.

23-27. *Mississippi River at Alton.*—A cross-section of the river was

taken opposite the water works above the city of Alton and about fifteen miles below the mouth of the Illinois, with the aim of determining the extent of commingling of the Illinois and Mississippi. Five samples were taken at about equidistant points.

28. *Missouri River at West Alton*.—The condition of the Missouri River just before its union with the Mississippi is shown by the collection at this point.

29-32. *Mississippi River at the Chain of Rocks*.—A cross-section of four samples was taken directly opposite the intake of the St. Louis Water Works, four miles below the mouth of the Missouri. The extent of commingling of the Missouri and Mississippi at this point is well shown by the chlorines.

33. *St. Louis Tap Water*.—The water drawn from the river at the Chain of Rocks is passed through several settling basins before delivery to the consumers. The grosser effect of subsidence is shown by the reduction of the suspended solids from 1699.5 parts per million (26 determinations) at the inlet tower at the Chain of Rocks to 90.6 (25 determinations) in the tap water.

34-38. *Mississippi River at Jefferson Barracks*.—A cross-section was taken six miles below the city of St. Louis for the purpose of determining the effect produced upon the Mississippi River by the addition of the sewage and manufacturing waste of the city.

In all cases the collection was made regularly from a definite position in the river and this point was fixed with a view to avoiding local sources of contamination. All of the collecting stations, except Kampsville, were visited prior to the opening of the investigation and reliable persons chosen for regular collectors. Explicit instruction was given concerning the methods of collection, and, in addition, printed directions were sent out weekly with each collecting bottle. Each bacterial sample was collected in a 4-ounce glass bottle, which was sterilized in the laboratory, enclosed in a tight-fitting metal case, which was itself placed in a large packing canister, ten inches deep and six and one-half inches in diameter, and the whole, together with the corresponding bottle for the chemical sample, fitted into a wooden box, one such outfit being shipped to the collector each week. Great pains were taken to ensure that the sample was obtained with due bacterial precautions, and, in order to emphasize the instructions given to the collector, personal visits, involving careful supervision and recurrent demonstration, were made frequently to all the more important points of collection. The water was always taken at a point about eight inches below the surface and in midstream except at Joliet and save where cross-sections were taken.

The bottle of water, when collected, was placed in its covered case and this case was then packed in the canister and completely surrounded with ice. The hour of collection was so timed as to permit of as speedy shipment as possible.

Although every attempt was made to minimize the dangers arising from transportation of the sample, there can be no doubt that our work has suffered from the unavoidable delay in plating, and it must be freely admitted that the numerical counts obtained in the routine work have not strictly comparable values. The samples collected at the stations along the upper end of the river (as far down as La Salle) usually reached us on the day of collection, while the samples from a greater distance did not get to the laboratory until the day after. The ice in the packing cases usually remained unmelted when the outfit was not more than 24 hours in transit, but occasionally it happened that a case was delayed, or by the carelessness of express agents was exposed to the midsummer sun, and in such instances the ice melted and the temperature of the sample rose to nearly the atmospheric temperature. Note was always taken of the condition of the sample on its arrival and when prolonged delay had occurred the sample was discarded.

Even when the shipping case arrived speedily and in good condition, however, a serious source of error existed. It has already been shown elsewhere (Jordan and Irons, 1899) that the number of bacteria in a bottle of water packed in ice (temperature 2° - 5° C.) sometimes increases and sometimes diminishes, and it has been pointed out that when the initial temperature of the water is high (*i. e.*, over 18° or 20°) a destruction of bacterial life takes place if the water be suddenly chilled; while on the other hand if the initial temperature of the water be but slightly above freezing-point, ice-packing and shipment under ordinary conditions is followed by an extensive multiplication of the water bacteria. In this respect, therefore, the results obtained at different seasons of the year are not strictly comparable, since the initial temperature of the water has varied from 0° C. to over 30° C. during the period covered by our analyses. The numbers reported for the summer months are doubtless somewhat too low, while those for the winter are unquestionably too high. It must be remembered, however, that while it is true that such changes in the bacterial population of the water often take place during transportation, these changes are rarely, if ever, great enough to alter the relative position of waters which, at the outset, are widely apart. The bacterial content of a badly polluted water is never depressed to the level of a pure water, and, on the other hand, the multiplication taking place in a relatively pure water is never so great, under

the conditions in which we have worked, as to cause it to rank with the more seriously polluted waters. Taking into consideration all the disturbing and variable factors, such as the effect of ice-packing, delay in transportation and original differences in the water itself, it is truly remarkable that the series from many of the collecting stations (*e. g.*, those from the Illinois River at Averyville and the Sangamon River at Chandlerville) should show so great uniformity as is the case.

Throughout the investigation, attempt has been made to control the sources of error incident to transportation by plating as many samples of water as possible at the places of collection. This has necessitated a number of visits to the more important collecting stations, and it is believed that the exact comparison thereby made possible is of considerable value. The results of this special work will be detailed elsewhere (p. 304).

The culture medium used for plating has been standard nutrient agar prepared with fresh beef juice and one per cent Witte's peptone. The agar has been made up on the basis of a —1.0 reaction (*i. e.*, the medium, after being rendered neutral to phenolphthalein, has had 10 cc. of $\frac{n}{1}$ HCl added to each litre). Preliminary experiments⁴ indicated that somewhat larger counts were obtained with this degree of acidity than with —1.5, and while experience gathered during the course of the investigation has shown that this is not true at all times and of all the waters studied, the use of a medium of uniform reaction possesses unmistakable advantages, and for purposes of comparison is indispensable. The choice of an agar rather than of a gelatin medium was dictated solely by the conditions governing the investigation. The necessity of sometimes plating in out-of-the-way localities and at high summer temperature prohibited the general use of gelatin and it was thought desirable to employ one medium throughout. It is of course recognized that slightly lower counts are obtained with agar than with gelatin, but in the well-known lack of any medium capable of affording exact information as to the living bacterial population of a water, relative and comparable results are at present all that can be aimed at.

Reference has been made elsewhere (Jordan and Irons, 1899) to the importance of a uniform method of dilution. We have endeavored to regulate the dilution so that no more than 100 colonies appear on the plate to be counted, and it is believed that the relative accuracy of the

¹ For example: Lake Michigan water with—

Neutral agar gave on the average of a series 1425 colonies.

10 acid	"	"	"	"	"	"	3550	"
15 "	"	"	"	"	"	"	2450	"

results is greatly enhanced by this method, since the inhibition and obscuration of colonies are thereby minimized. The plates have been regularly incubated in a dark room (temperature 20°-32° C.) for eight days before counting; the atmosphere of the room has been kept suitably moist.

TABLE I.—ILLINOIS AND MICHIGAN CANAL.

<i>Bridgeport.</i>			
Date.	Chlorine. (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 29.....	237	8	1310000
July 3.....	78	..	225000
10.....	128	16	445000
17.....	97	17	1415000
24.....	112	23	1070000
Aug. 6.....	76	19	515000
Sept. 11.....	117	12	950000
18.....	121	14	540000
25.....	161	13	1190000
Oct. 2.....	108	13	1370000
9.....	74	11	600000
30.....	99	12	1360000
Nov. 6.....	74	9	1700000
13.....	126	6	1850000
20.....	156	5	2650000
27.....	106	10	1680000
Dec. 4.....	102	8	Not plated.
11.....	135	9	630000
18.....	118	9	970000
25.....	166	6	3190000

TABLE II.—ILLINOIS AND MICHIGAN CANAL.

<i>Lockport.</i>			
Date.	Chlorine. (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 22.....	98	..	1115000
29.....	184	..	1055000
June 5.....	132	..	360000
12.....	101	..	1230000
19.....	121	24	300000
26.....	103	21	570000
July 3.....	105	22	180000
10.....	101	21	185000
17.....	119.5	22	935000
24.....	106	24	520000
31.....	108	22	1210000
Aug. 7.....	135	21	100000
21.....	109	22.5	95000
28.....	123	24	40000
Sept. 4.....	105	23	80000
11.....	130	18	920000
18.....	110	20	260000
25.....	132	16	220000
Oct. 2.....	11	740000
16.....	91	14	310000
23.....	115	18	300000
30.....	110	12	690000
Nov. 6.....	120	9	1040000
13.....	121	10	2060000
20.....	128	12	1340000
27.....	130	9	510000
Dec. 4.....	114.5	..	350000
11.....	114	10	650000
18.....	114	8	510000
26.....	178	7	1650000

TABLE III.—DESPLAINES RIVER.

		<i>Lockport.</i>		No. of colonies per cc.
Date.		Chlorine, (Pts. per million.)	Temperature of water, °C.	
May	22.....	3.4	..	4500
	29.....	2.7	..	3600
June	5.....	2.8	..	6950
	12.....	3.55	..	4200
	19.....	4	20	4500
	26.....	4	24	1250
July	3.....	4.9	25	6200
	10.....	4.9	24	6500
	17.....	3.65	24	5200
	24.....	7.7	26	11650
	31.....	5.5	23	8150
Aug.	7.....	6.6	23	4750
	21.....	10.2	24	2300
	28.....	9.3	25	44100
Sept.	4.....	7.4	21	2400
	11.....	8	20	9800
	18.....	7.8	18	19000
	25.....	7.2	16	2800
Oct.	2.....	7.2	12	16400
	8.....	8.6	12	4000
	16.....	8.6	20	8400
	23.....	7.4	20	11400
	30.....	8.1	14	6500
Nov.	6.....	11.2	9	8700
	13.....	12.8	8	3400
	27.....	18.1	8	3500
Dec.	11.....	8.9	9	12900
	18.....	10.9	9	34000

TABLE IV.—DESPLAINES RIVER.

		<i>Joliet (above town).</i>		No. of colonies per cc.
Date.		Chlorine, (Pts. per million.)	Temperature of water, °C.	
May	23.....	65	..	390000
	29.....	73	..	230000
June	5.....	48	..	530000
	12.....	92	..	1620000
	19.....	113	21	280000
	26.....	90	21	590000
July	3.....	112	22	135000
	10.....	107	21.5	115000
	17.....	80	21	575000
	24.....	90	23	60000
	31.....	105	22	20000
Aug.	7.....	103.5	21	70000
	21.....	104	24	305000
	26.....	112	22	400000
Sept.	4.....	116	20	140000
	11.....	114	11	600000
	18.....	107	18	90000
	25.....	121	15	230000
Oct.	92	20	590000
	23.....	109	13	630000
	30.....	127	..	450000
Nov.	13.....	112	..	870000
	21.....	144	..	1310000
	27.....	118	..	770000
Dec.	4.....	112	..	670000
	11.....	98	..	620000
	18.....	130	..	490000
	26.....	120	..	840000

TABLE V. DESPLAINES RIVER.

Juliet (below town).

Date.	Chlorine, (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 23	65	..	654000
29	74	..	610000
June 5	48	..	850000
12	94	..	760000
19	112	19	530000
26	87	21	180000
July 3	113	22.5	1760000
10	100	21	120000
17	82.5	22	830000
.....	90	25	170000
31	102	22	355000
Aug. 7	134.75	26	390000
21	115.5	23	2515000
28	117	23	370000
Sept. 4	128	22	2540000
11	112	17	280000
18	109	18	120000

TABLE VI.—KANKAKEE RIVER.

Wilmington.

Date.	Chlorine, (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 5	1.8	3	..	4700
12	1.65	2.5	..	3500
19	1.65	1.5	24	7650
26	1	24	2800
July 3	2	.8	25	2200
10	2.4	1	23	1900
17	3.3	1.5	22	5900
24	3.8	1.25	27	2100
31	3.4	1.25	24	4250
Aug. 7	3.4	1	22	1850
21	4.1	1.2	22	3650
28	3.7	.8	25	2600
Sept. 4	3.9	.8	23	1950
11	4.6	.8	19	1400
18	3.9	.8	20	15600
25	4.9	.8	11	4100
Oct. 2	3.8	.7	8	2100
9	3.8	.8	13	1700
16	3.8	.8	19	1500
23	4.6	.8	14	4600
30	3.8	.8	10	4000
Nov. 6	4.2	1.2	5	2100
13	3.6	1.5	5	6900
20	2.6	1.7	8	4400
27	3	1.5	5	2300
Dec. 11	3.3	1.7	6	5000
18	2.8	2	0	13500
26	2.7	1.7	5	25700

TABLE VII.—ILLINOIS RIVER.

Morris.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 29.....	42	7.3	21	782000
June 5.....	19.2	9.11	24	115000
13.....	39.1	6	26	720000
19.....	60.5	5.25	24	250000
27.....	48	5.66	24	640000
July 11.....	67	5.3	24	270000
17.....	37	7.5	24	30000
31.....	62	6	24	275000
Aug. 7.....	71.75	6.3	24	130000
21.....	77.5	5.1	25	65000
29.....	93.5	4.1	26	70000
Sept. 4.....	96	4.1	25	570000
12.....	98	5.3	20	1140000
19.....	91.5	5.2	13	96000
25.....	100	5.6	17	345000
Oct. 2.....	99	5.4	10	351000
7.....	66	5	13	604000
16.....	62	5.6	20	16000
23.....	75	5.6	15	434000
30.....	73	5.1	10	466000
Nov. 6.....	65	6	16	568000
14.....	50	6.3	8	372000
20.....	47	6.3	9	178000
27.....	70	6	6	234000
Dec. 26.....	50	..	14	115000

TABLE VIII.—FOX RIVER.

Ottawa.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 29.....	1.25	6	..	29500
June 5.....	2.2	4	29	27000
12.....	2.85	3	26	16500
19.....	2.85	4	28	4000
26.....	3.3	3	27	5000
July 3.....	2.9	3	28	8000
10.....	3.9	3	28	2500
17.....	5	5	26	34500
24.....	3.1	3	28	1300
31.....	5.1	3	25	1800
Aug. 7.....	5	4	27	1650
21.....	6	4	28	450
28.....	4.9	3	31	6400
Sept. 4.....	5.75	3.5	26	1200
11.....	5.1	4	25	1400
18.....	4.7	4	21	3700
25.....	6	4	18.5	3500
Oct. 2.....	5.2	4	16	2500
9.....	5.7	4	17.5	8700
16.....	6.2	4	17	1600
23.....	6	4.5	17.5	1500
30.....	7.4	4	13	1600
Nov. 6.....	6.2	4	9	1700
13.....	5.4	3.5	9.5	1000
20.....	5.7	3.5	13	3700
27.....	7.6	3.5	7	3200
Dec. 11.....	6.4	4	10	6300
21.....	7.4	4	2	3200
28.....	5	3.5	0	5500

TABLE IX.—ILLINOIS RIVER.

Ottawa (above town).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 19	25.5	4.5	26	60000
26	37.4	4	26	15000
July 3	43	3.5	27	50000
10	62	4	27	25000
17	43.5	6	25	65000
24	35	4	29	8000
31	54	4	26	21000
Aug. 7	53	3	28	2650
21	66	3	27	2750
28	77.5	4	30	650
Sept. 4	47.8	3	25	9500
11	94	3.5	24	38300
18	91	3.5	20.5	17400
25	81.5	3	18	13400
Oct. 2	95	3	15	7300
9	85.5	3	16	2800
16	69	3	18	7300
23	69	3	18	5200
30	61	3.5	13.5	8200
Nov. 6	64	3	8	52000
13	42	3	9	1300
24	81	3	13	9000
27	46	3	7	25500
Dec. 11	44	3	11	108000
21	34	3.5	2	29000
28	22	3	0	130000

TABLE X.—BIG VERMILION RIVER.

La Salle.

Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.	Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.
May 24	9	4200	Sept. 12	80	1400
30	13.4	1680	19	84	1600
June 6	6.55	6250	26	131	1700
15	16.6	5100	Oct. 3	127	3700
20	8.8	1550	10	118	3900
27	18.55	1100	17	123	4700
July 4	29.9	2450	24	109	3700
11	8.1	3100	31	114	1800
18	9.25	8150	Nov. 7	118	2500
25	21.5	1850	21	82	47300
Aug. 1	34.4	1700	28	104	5800
8	48.1	1800	Dec. 5	113.5	10500
22	35	1050	12	80	23000
29	53	10100	19	30	12600
Sept. 5	66	2800	26	12	62000

TABLE XI.—ILLINOIS RIVER.

La Salle.

Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.	Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.
May 24.....	15	11100	Sept. 19.....	71.5	2900
30.....	12	25000	26.....	72	700
June 6.....	8.7	6000	Oct. 3.....	82	3600
15.....	22.2	2600	10.....	74	6800
20.....	22.1	14500	17.....	67	16400
27.....	40	4900	24.....	58	10400
July 4.....	30.7	7000	31.....	52	5600
11.....	63	7150	Nov. 7.....	60	7500
18.....	29	11850	14.....	34	14200
25.....	30	2000	21.....	33	7900
Aug. 1.....	47.5	1650	28.....	37	6600
8.....	48	1800	Dec. 5.....	43.5	27500
22.....	63.5	1600	12.....	39	14200
29.....	67.5	6900	19.....	31	228000
Sept. 5.....	77.5	1100	26.....	20	32400
12.....	69	800			

TABLE XII.—ILLINOIS AND MICHIGAN CANAL.

La Salle.

Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.	Date.	Chlorine. (Pts. per million.)	No. of colonies per cc.
May 24.....	12	20000	Sept. 12.....	16.5	104000
30.....	13.3	30000	19.....	20	62000
June 6.....	11.5	22500	26.....	21	36000
15.....	9.8	20000	Oct. 3.....	18	22000
20.....	11.3	13000	10.....	14	34000
27.....	8.75	91500	17.....	15	61000
July 4.....	8.7	16500	24.....	14	33000
11.....	12	23000	31.....	17.5	48000
18.....	16.3	19500	Nov. 7.....	15	16000
25.....	15	42000	21.....	12	152000
Aug. 1.....	13	24000	28.....	13	52000
8.....	12	74500	Dec. 5.....	15.5	84000
22.....	13	24350	12.....	26	147000
29.....	13	47750	19.....	33	107000
Sept. 5.....	15	19000	26.....	40	39000

TABLE XIII.—ILLINOIS RIVER.

Henry.

Date.	Chlorine. (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 30.....	12.6	..	14500
June 6.....	12.6	29	26000
12.....	16.4	29	8500
20.....	20.4	30	7000
27.....	35.1	28	11500
July 5.....	26.1	29	27000
12.....	48.5	30	30500
26.....	27.5	31	43000
Aug. 2.....	37.5	29	16500
8.....	44	26	4500
22.....	54.5	28	3000
29.....	59	28	500

TABLE XIII.—ILLINOIS RIVER. *Continued.*

<i>Henry.</i>				
Date.	Chlorine, (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.	
Sept. 6	56	29	10600	
12	60	20	5200	
13	68	21	2900	
26	68	18	1100	
Oct. 3	66	15	3100	
10	75.5	16	7200	
17	67	13	6800	
25	61	14	2900	
31	53	10	2500	
Nov. 7	53	9	800	
15	45	9	4000	
22	36.5	8	4400	
28	35	8	2700	
Dec. 5	41.5	3	4700	
12	44	2	16300	
19	34	0	74000	
27	20	0	49600	

TABLE XIV.—ILLINOIS RIVER.

<i>Averyville.</i>				
Date.	Chlorine, (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 30	30	30.40	..	2460
June 8	15.4	31.80	25	3450
12	11.5	30.70	26	5200
20	13.7	29.40	26	950
27	19.4	28	26	3550
July 3	18	27.10	26	7750
10	33.75	27.70	26	1050
18	44	29.10	26	3000
25	29	28.75	28	3200
Aug. 1	34	27.60	25	3500
8	29	27.60	24	1100
22	40.5	26.65	26	1350
29	45	26.70	28	2000
Sept. 5	51	27	27	7300
12	51	27.25	21	2000
19	53	27.50	20	1400
26	56	27.35	12	1600
Oct. 3	65	27.25	12	600
10	67	27.22	15	1800
17	66	27.55	18	2800
24	69	27.50	15	3400
31	63	27.80	12	500
Nov. 7	54	27.80	7	2300
14	51	28.15	9	3000
21	47	28.25	10	19500
28	32	28.10	6	1500
Dec. 5	34	27.90	3	1500
12	38	28.20	5	3700
19	40	28.90	1	11600
26	31	29.80	0	7000

TABLE XV.—ILLINOIS RIVER.
Wesley City.

Date.	Chlorine. (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 20.....	12.85	..	932000
June 7.....	16.5	..	440000
12.....	13.9	..	275000
19.....	14	..	2785000
26.....	18.7	..	3390000
July 3.....	18.6	..	1975000
10.....	33.75	..	275000
24.....	28.5	..	95000
Aug. 21.....	41.5	..	710000
28.....	43	..	205000
Sept. 5.....	46	38	170000
12.....	49	20	240000
26.....	53	15	5000
Oct. 3.....	53	12	1000000
10.....	67	12	830000
31.....	67	10	2680000
Nov. 7.....	59	6	80000
15.....	52	10	520000
22.....	52	0	5000
Dec. 5.....	35	..	20000
13.....	38	..	40000
20.....	37	..	5000

TABLE XVI.—ILLINOIS RIVER.
Pekin.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	15.8	3.5	18	120000
30.....	15	3.6	18	542000
June 6.....	14	4.7	26.25	129000
13.....	12.6	3.5	25	205000
21.....	14.6	2	26	225000
27.....	15.3	1	27	2030000
July 6.....	17.8	2	24	520000
12.....	34.25	1	28	1435000
18.....	40	2	26	470000
25.....	27.5	1.5	30	980000
Aug. 1.....	34	1	25	1940000
8.....	27	1	21	985000
22.....	41.5	0.5	26	10000
29.....	43	1	29	30000
Sept. 5.....	48	1	28	650000
12.....	50	1	20	310000
18.....	42.5	1.5	20	240000
26.....	53	1	15	120000
Oct. 3.....	61	1	12	500000
10.....	55	1	12	430000
31.....	66	1.5	10	30000
Nov. 7.....	58	1	6	150000
15.....	51	2	10	30000
22.....	48	1.5	0	1650000
30.....	35	1.5	0	380000
Dec. 5.....	34	0.5	..	140000
13.....	36.5	2	..	10000
21.....	38	2	..	5000
28.....	34	2.5	..	20800

TABLE XVII.—ILLINOIS RIVER.

Havana.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 30.....	13.6	8.7	21	4500
June 6.....	13	9.3	26	18450
13.....	13.5	8.8	25	15900
20.....	12.1	7.5	26	2500
28.....	14.9	5.2	25	4500
July 5.....	14.7	4.7	26	2400
12.....	23	4.1	26	7300
19.....	36	4.9	27	5700
26.....	31	4.8	30	850
Aug. 9.....	27.5	4.1	26	1550
23.....	34	3	26	900
30.....	39	2.2	29	9800
Sept. 6.....	40	2.4	29	1900
14.....	46	2.5	22	1500
20.....	35	3.5	14	3400
27.....	49	3	16	3700
Oct. 4.....	52	3	14.5	2500
11.....	59	3.2	16	6600
18.....	58	3.2	17	8800
25.....	60.5	3	12	3900
Nov. 1.....	63	3.5	9	7000
15.....	51	3.7	10	3300
22.....	47.5	4.4	11	128000
29.....	43	4	6	41600
Dec. 6.....	35	3.1	2	85000
20.....	35	4.8	1	66800

TABLE XVIII.—SANGAMON RIVER.

Chandlerville.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 31.....	2.35	8	33	8400
June 6.....	3.2	8	26.5	11600
21.....	3.3	5	27	1830
July 5.....	3.6	4	27	3100
12.....	4	4	27	8350
Aug. 2.....	4	3	26	2100
9.....	4.9	3.5	25	9900
31.....	4.5	" Very low."	27	1200
Sept. 13.....	4.3	" "	22	1900
20.....	4.7	" "	19	11500
28.....	3.3	" "	7	4200
Oct. 4.....	4.6	" "	6	1300
18.....	3.1	" "	12	2000
25.....	4.4	" "	18.5	3200
Nov. 1.....	4.9	" "	10	1500
8.....	5.8	1	10	10800
22.....	6	2	12	7900
29.....	..	1	7	2500
Dec. 6.....	6	1	3	1200
13.....	5.1	3	4	6400
20.....	5.4	2.5	2	5900

TABLE XIX.—ILLINOIS RIVER.

Beardstown.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	10.15	10.04	..	9300
June 1.....	7	11.08	..	5150
19.....	9	9.4	26	5500
July 6.....	10.05	7.1	25	7800
13.....	16	6.7	29	4700
27.....	33	6.8	21	1700
Aug. 3.....	20	6.4	26	5450
10.....	23	7.9	21	3000
24.....	19.2	6.25	21	4200
31.....	28	6.75	20	2000
Sept. 7.....	28	6.2	26	8500
14.....	25	6.2	16	1500
28.....	32.5	6.4	16	3700
Oct. 5.....	38.5	6.2	13	3900
12.....	45	6.3	17	3500
19.....	40	6.65	18	8500
26.....	49	6.5	19	2600
Nov. 2.....	49	6.7	2	4800
9.....	52	6.63	8	23800
16.....	46	6.8	12	2400
23.....	37	7.25	10	7400
30.....	39	6.6	6	26400
Dec. 7.....	30	6.45	4	21900
14.....	26.5	7	0	65000
21.....	30	7.7	-1	120000
28.....	32	7	-1	12500

TABLE XX.—ILLINOIS RIVER.

Kampsville.

Date.	Chlorine. (Pts. per million.)	Temperature of water, °C.	No. of colonies per cc.
May 31.....	3.8	22	8490
July 5.....	8.1	27	7750
19.....	17.5	29	600
26.....	30	28	900
Aug. 2.....	21	28	2950
24.....	15.8	28	550
Sept. 6.....	19	29	710
13.....	13	22	340
20.....	17	19	4400
27.....	24	17	3300
Oct. 11.....	29	17	520
18.....	36	20	5100
27.....	36	18	1300
Nov. 4.....	39	12	2300
15.....	41	12	1800
22.....	34.5	12	19100
28.....	31	11	4300
Dec. 6.....	32.5	5	3500
20.....	19	2	23500

TABLE XXI.—ILLINOIS RIVER.
Grafton.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	6.8	17.5	..	1690
31.....	3.7	16.1	..	5730
June 7.....	5.1	16.7	22	17000
14.....	7.35	14.1	26	97000
21.....	5.6	26	30000
July 5.....	7.5	17.6	26.5	9000
12.....	9	13.2	29	6000
19.....	11.8	9.7	33	4000
26.....	21	7.6	34	11500
Aug. 9.....	12.7	7.6	25	15050
21.....	14.6	4.2	31	1400
30.....	13.4	3.3	30	1400
Sept. 6.....	19	3.4	35	1330
13.....	17.8	4.4	24	180
20.....	21.2	4.8	20	1340
27.....	22.5	4.7	19	1500
Oct. 4.....	19.1	18	1200
11.....	27.8	3.2	17	800
25.....	29	2.75	19	440
Nov. 1.....	27.5	3.4	18	1500
8.....	40	5.4	9	830
15.....	40	6.2	10	580
22.....	30	6	13	3600
29.....	26	5.9	8	5200
Dec. 2.....	32.5	4.3	3	1390
13.....	25	4.3	3	2200
20.....	4.4	2	29100
27.....	22	2.1	0	35000

TABLE XXII.—MISSISSIPPI RIVER.
Grafton.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	1.6	17.5	..	8000
31.....	.2	16.1	23	8940
June 7.....	1.6	16.7	23	26000
14.....	1.8	14.1	24	31000
21.....	1.5	27	16000
28.....	1.3	26.5	9000
July 5.....	.6	14.6	26	5000
12.....	1.7	13.2	27	15000
19.....	1.7	9.7	28	6500
26.....	2.1	7.6	30	1500
Aug. 9.....	2.9	7.6	26	4300
21.....	3.1	4.2	26	900
30.....	3	3.3	28	1400
Sept. 6.....	2.7	3.4	30	1300
13.....	2.6	4.4	23	1800
20.....	2.8	4.8	20	2000
27.....	2.5	4.1	18	2200
Oct. 4.....	2.6	16	4500
11.....	3	3.2	16	1500
25.....	3.2	2.75	18	1700
Nov. 1.....	3.5	3.4	16.5	2600
8.....	3	5.4	9	5800
15.....	2	6.2	10	5400
22.....	2	6	12	6300
29.....	3.6	5.9	7	1700
Dec. 6.....	2.2	4.3	3	1100
13.....	3.4	4.3	3	800
20.....	3.8	4.4	1	3100
27.....	18.6	2.1	0	45000

TABLE XXIII.—MISSISSIPPI RIVER.

Alton (east bank).

Date.	Chlorine, (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 21.....	6.6	17.50	16	3470
31.....	3	16.50	21	17400
June 7.....	3.6	16.80	24	25000
14.....	6.2	17	19	14500
21.....	3.9	14.40	19	11000
28.....	2.7	13.50	27	39000
July 5.....	2.8	15.30	26	5000
12.....	4.35	15.80	29	5500
19.....	4.65	12.20	28	3000
26.....	9.5	9	30	5500
Aug. 2.....	15.7	7.20	29	5500
9.....	12.2	7	26	9500
30.....	6.6	3.1	28	1200
Sept. 6.....	7.2	2.9	30	900
20.....	9.6	4	20	3000
27.....	7.2	3.8	18	2300
Oct. 4.....	5.7	2.8	15	2200
11.....	6.5	2.2	16	1700
.....	9.55	1.9	18	2600
Nov. 1.....	11.4	2.3	12	6250
15.....	10.8	5.4	10	2700
29.....	15.4	3.8	8	1800
Dec. 13.....	10.5	3	4	7800
20.....	9.9	3	0	12700

TABLE XXIV.—MISSISSIPPI RIVER.

Alton (east of centre).

Date.	Chlorine, (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 21.....	2.6	17.50	18	5800
31.....	3.3	16.50	21	9500
June 7.....	1.7	16.80	24	24500
14.....	2.3	17	19	18000
21.....	2	14.40	19	13000
28.....	1.6	13.50	27	7000
July 5.....	1.1	15.30	26	8000
12.....	2	15.80	29	7000
19.....	3.45	12.26	28	3500
26.....	4.8	9	30	7500
Aug. 2.....	5.8	7.2	29	2500
9.....	7.5	7	26	4500
30.....	5.6	3.1	28	1250
Sept. 6.....	5.2	2.9	30	900
20.....	7.8	4	20	5400
27.....	5.4	3.8	18	2200
Oct. 4.....	5	2.8	15	1900
11.....	6.3	2.2	16	1300
.....	9.6	1.9	18	2100
Nov. 15.....	7	5.4	10	1700
29.....	10.2	3.8	8	1900
Dec. 20.....	9.4	3	0	40400

TABLE XXV.—MISSISSIPPI RIVER.

Alton (midstream).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	2.1	17.50	18	7160
31.....	1.7	16.50	21	13750
June 7.....	1.3	16.80	24	27500
14.....	1.5	17	19	22500
21.....	1.6	14.40	19	7000
28.....	.75	13.50	27	6500
July 5.....	.8	15.30	26	8000
12.....	2.25	15.80	29	10000
19.....	2.15	12.20	28	9000
26.....	2.5	9	30	6000
Aug. 2.....	2.7	7.2	29	1000
9.....	3.1	7	26	3000
30.....	4.5	3.1	28	700
Sept. 6.....	4.1	2.9	30	900
20.....	5.2	4	20	1300
27.....	3.5	3.8	18	2200
Oct. 4.....	3.8	2.8	15	2500
11.....	4.1	2.2	16	1700
.....	6.6	1.9	18	1600
Nov. 1.....	8.1	2.3	12	6100
29.....	7.2	3.8	8	3000
Dec. 13.....	6	3	4	2000
20.....	6.5	3	0	9500

TABLE XXVI.—MISSISSIPPI RIVER.

Alton (west of centre).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 24.....	1.4	17.50	21	6500
31.....	1.8	16.50	21	10750
June 7.....	1.2	16.80	24	25500
14.....	1.6	17	19	19500
21.....	1.7	14.40	18	15000
28.....	1.2	13.50	27	9500
July 5.....	1.1	15.80	26	9000
12.....	2.1	15.80	29	8500
19.....	2.2	12.20	28	9500
26.....	2.6	9	30	5000
Aug. 2.....	2.6	7.2	28	2500
9.....	3	7	26	3000
30.....	3.4	3.1	28	700
Sept. 6.....	3.4	2.9	30	800
20.....	4.6	4	20	3300
27.....	2.4	3.8	18	2900
Oct. 4.....	2.2	2.8	15	1650
11.....	3.2	2.2	16	1400
.....	3.8	1.9	18	1600
Nov. 1.....	5	2.3	12	3700
15.....	2	5.4	10	2800
29.....	3.2	3.8	8	2100
Dec. 13.....	3.2	3	4	800
20.....	4.3	3	0	6300

TABLE XXVII.—MISSISSIPPI RIVER.

Alton (west bank).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 21.....	1.5	17.50	18	11700
31.....	1.75	16.50	21	17500
June 7.....	1.2	16.80	24	37500
14.....	1.8	17	19	24500
21.....	1.6	14.40	19	16000
28.....	.65	13.50	27	5500
July 5.....	.8	15.3	26	10500
12.....	2.55	15.8	29	8000
19.....	2.55	12.2	28	6000
26.....	2.7	9	30	11000
Aug. 2.....	2	7.2	28	3000
9.....	2.95	7	26	1000
30.....	3.4	3.1	28	650
20.....	3	4	20	2900
27.....	2.7	3.8	18	1500
Oct. 4.....	2.7	2.8	15	2000
.....	6.6	1.9	18	1400
Nov. 1.....	3.8	2.9	12	900
15.....	1.8	5.4	10	3400
29.....	3.2	3.8	8	1200
Dec. 13.....	2.8	3	4	2200
20.....	4	3	0	2100

TABLE XXVIII.—MISSOURI RIVER.

W. Alton, Mo.

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
July 27.....	5.5	13.3	29	8000
Aug. 17.....	7.6	8.3	28	4000
Sept. 7.....	8.9	7.3	29	3750
14.....	10.6	6.7	22	12800
21.....	12.4	6.1	20	5000
28.....	13.6	5.5	18	4600
Oct. 5.....	15.3	5	17	7500
12.....	15.6	4.8	22	2100
19.....	13.9	4.6	18	20900
26.....	17.4	4.5	19	4000
Nov. 2.....	16	4.7	11	10600
16.....	27	4.4	12	8000
30.....	16.8	5	10	6900
Dec. 7.....	17.4	5	10	7700
14.....	18.4	6.8	0	6600
21.....	18.8	4.9	2	7700
28.....	24.4	3.2	1	18200

TABLE XXIX.—MISSISSIPPI RIVER.

Chain of Rocks (east bank).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 25.....	2.6	25	..	10650
June 1.....	3.1	22.7	22.5	16750
15.....	5.1	24.8	24.5	113000
22.....	4	28.8	26	23500
29.....	2.5	20.4	21	16000
July 13.....	2.6	23.9	27	32000
20.....	4.1	20.6	26	18000
27.....	3.5	16.6	29	2000
Aug. 3.....	5.1	14.3	29	7000
10.....	5.6	12.9	26	4100
24.....	5.3	9.7	27	1750
31.....	5.9	7.5	27	3000
Sept. 7.....	5.6	6.6	29	1300
14.....	4.6	6.6	22	1500
21.....	6.7	6.6	19	3400
28.....	5.2	5.3	16	1800
Oct. 5.....	2.5	4.5	15	2600
26.....	7.4	3	17	1500
Nov. 2.....	8.3	3.9	9	4000
13.....	6.2	6.2	9	5200
30.....	8.4	5.5	8	1800
Dec. 15.....	7.8	4.6	0	3700

TABLE XXX.—MISSISSIPPI RIVER.

Chain of Rocks (midstream).

Date.	Chlorine. (Pts. per million.)	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 25.....	2.75	25	..	16100
June 1.....	3	22.7	22	12400
15.....	4.1	24.8	24.5	68000
22.....	3.8	20.8	26	26000
July 13.....	3.2	23.9	27	23500
20.....	4.6	20.6	26	18500
27.....	4.1	16.6	29	8500
Aug. 3.....	5.1	14.3	29	12000
10.....	4.9	12.9	26	4400
24.....	5.8	9.7	27	3650
31.....	6.6	7.5	27	5200
Sept. 14.....	6.2	6.6	22	5100
21.....	8.1	6.6	19	3900
Oct. 20.....	12	3.3	17	5200
26.....	13.8	3	17	1600
Nov. 2.....	13.4	3.9	9	5200
13.....	12	6.2	9	4400
30.....	13.3	5.5	8	7300
Dec.	14	4.6	0	4500

TABLE XXXI.—MISSISSIPPI RIVER.

Chain of Rocks (Inlet Tower, St. Louis Water Works).

Date.	Chlorine, (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 25.....	3.3	25	..	11400
June 1.....	3.7	22.7	22	14300
15.....	3.9	24.8	24.5	69000
22.....	3.5	20.8	26	24000
29.....	2.65	26.4	24	27000
July 13.....	3.2	23.9	27	29000
20.....	4.5	20.6	26	12000
27.....	4.6	16.6	29	8500
Aug. 3.....	5.2	14.3	29	13000
10.....	4.7	12.9	26	5150
24.....	7.1	9.7	27	6550
31.....	7.1	7.5	27	4450
Sept. 7.....	7.8	6.6	29	3000
14.....	8.8	6.6	22	11000
21.....	9.7	6.6	19	4500
28.....	10.7	5.3	16	8900
Oct. 5.....	10.8	4.5	15	8900
20.....	12.2	3.3	17	7800
26.....	13.9	3	17	3800
Nov. 2.....	12.1	3.9	9	5100
13.....	15.6	6.2	9	1800
30.....	13.9	5.5	8	9400
.....	15.6	4.6	0	8500

TABLE XXXII.—MISSISSIPPI RIVER.

Chain of Rocks (west bank).

Date.	Chlorine, (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
May 25.....	3.95	25	..	20700
June 1.....	4.2	22.7	22	11400
15.....	4.3	24.8	24.5	57500
22.....	3.5	20.8	26	10500
29.....	2.85	20.4	24	11500
July 13.....	4.3	23.9	27	33000
20.....	4.6	20.6	26	14000
27.....	4.7	16.6	29	10500
Aug. 3.....	5	14.3	29	16000
10.....	5.1	12.9	26	2700
24.....	6.7	9.9	27	5150
31.....	7.2	7.5	27	8400
Sept. 14.....	10.1	6.6	22	7300
21.....	11.7	6.6	19	5900
28.....	10.9	5.3	16	7100
Oct. 5.....	13.5	4.5	15	4300
20.....	16.7	3.3	17	6000
26.....	14.4	3	17	2800
Nov. 2.....	12.5	3.9	9	4400
13.....	21.2	6.2	9	3200
30.....	15.7	5.5	8	7200
.....	17.9	4.6	0	13500

TABLE XXXIII.
St. Louis (tap water).

Date.	Chlorine, (Pts. per million).	No. of colonies per cc.	Date.	Chlorine, (Pts. per million).	No. of colonies per cc.
June 6.....	4.5	3150	Aug. 31.....	7.1	600
23.....	3.3	6500	Sept. 28.....	9.6	1400
July 6.....	8000	Oct. 12.....	11.6	670
14.....	4.8	8000	19.....	11.7	2790
21.....	4.6	1500	26.....	13.8	600
27.....	5.9	4500	Nov. 3.....	12.5	730
Aug. 3.....	5.2	7000	23.....	15.3	930
11.....	6.2	1600	Dec. 7.....	15.1	290
23.....	6	700	21.....	15.2	340

TABLE XXXIV.—MISSISSIPPI RIVER,
Jefferson Barracks (east bank).

Date.	Chlorine, (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 6.....	3.7	23.5	26	24100
16.....	4.2	22.8	26	69000
22.....	3.5	22	28	53000
29.....	2.85	20.5	26	4000
July 6.....	2.8	22.9	26	14000
14.....	3.7	23.6	28	21500
21.....	5.7	19.8	28	9000
27.....	4.8	16.2	30	13000
Aug. 3.....	6	14.3	32	15500
11.....	4.7	14.4	29	9700
23.....	6.1	8.6	30	6750
31.....	6.9	7.5	30	3200
Sept. 22.....	6.7	6.7	19	37800
28.....	6	5.3	17	16100
Oct. 12.....	6.4	3.5	20	20600
19.....	8.2	3.2	19	31100
26.....	...	2.8	19	19200
Nov. 3.....	6.7	4	9	2600
23.....	7.8	6.6	12	3600
Dec. 7.....	8.2	4.8	3	1000
14.....	6.8	4.6	2	2000
21.....	9	5.3	0	11900

TABLE XXXV.—MISSISSIPPI RIVER,
Jefferson Barracks (east of centre).

Date.	Chlorine, (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 6.....	4	23.5	26	22650
16.....	4	22.8	26	25000
23.....	3.4	22	28	26500
29.....	2.9	20.5	26	18500
July 6.....	2.9	22.9	26	12000
14.....	3.7	23.6	28	34000
21.....	5	19.8	28	17000
Aug. 3.....	5.3	14.3	32	7500
11.....	4.7	14.4	29	12100
23.....	6.1	8.6	30	6800
31.....	6.2	7.5	30	2200
Sept. 8.....	6	6.3	30	42000
22.....	6.6	6.7	19	8400
28.....	6.3	5.3	17	10600
Oct. 12.....	6.4	3.5	20	14000
19.....	8	3.2	19	6000
26.....	8.2	2.8	19	6600
Nov. 3.....	7.4	4	9	2800
23.....	7.6	6.6	12	4500
Dec. 7.....	8	4.8	3	1700
14.....	7.6	4.6	2	1700
21.....	9.5	5.3	0	6000

TABLE XXXVI.—MISSISSIPPI RIVER.

Jefferson Barracks (midstream).

Date.	Chlorine. (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 6.....	4	23.5	26	15400
16.....	4.6	22.8	26	17500
22.....	2.5	22	28	56500
29.....	3	20.5	26	43000
July 6.....	2.75	22.9	26	14500
14.....	3.9	23.6	28	21000
21.....	5.1	19.8	28	17000
27.....	5.2	16.2	30	8000
Aug. 3.....	5.6	14.3	32	14500
11.....	5	14.4	29	4400
23.....	6.1	8.6	30	3550
31.....	6.4	7.5	30	5100
Sept. 8.....	7	6.3	30	10100
22.....	8	6.7	19	20400
28.....	7.2	5.3	17	4900
Oct. 12.....	8	3.5	20	5200
18.....	10.2	3.2	19	14100
26.....	9.8	2.8	19	5800
Nov. 3.....	8.3	4	9	5900
23.....	6.8	6.6	12	4000
Dec. 7.....	8	4.8	3	3100
14.....	8.2	4.6	2	2700
21.....	11.6	5.3	0	9800

TABLE XXXVII.—MISSISSIPPI RIVER.

Jefferson Barracks (west of centre).

Date.	Chlorine. (Pts. per million).	State of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 6.....	4.15	23.5	26	26250
16.....	3.5	22.8	26	18000
22.....	3.6	22	28	12000
29.....	2.9	20.5	26	40000
July 6.....	2.6	22.9	26	21500
14.....	4.3	23.6	28	31000
21.....	5.6	19.8	28	27000
27.....	4.6	16.2	30	9500
Aug. 3.....	5.3	14.3	32	34000
11.....	5.9	14.4	29	13000
23.....	6.6	8.6	30	9650
31.....	6.6	7.5	30	22900
Sept. 8.....	7.4	6.3	30	37800
22.....	9.6	6.7	19	91600
28.....	9.2	5.3	17	9700
Oct. 12.....	10.4	3.5	20	14700
19.....	9.7	3.2	19	27800
26.....	12.4	2.8	19	22100
Nov. 3.....	12.8	4	9	10000
23.....	11	6.6	12	12400
Dec. 7.....	13.1	4.8	3	3700
14.....	11.8	4.6	2	3200
21.....	13.6	5.3	0	4800

TABLE XXXVIII.—MISSISSIPPI RIVER.

Jefferson Barracks (west bank).

Date.	Chlorine. (Pts. per million).	Stage of river in feet.	Temperature of water, °C.	No. of colonies per cc.
June 6.....	4.45	23.5	26	24250
16.....	3.5	22.8	26	27000
22.....	2.9	22	28	27000
29.....	3	20.5	26	18000
July 6.....	3.1	22.9	26	42000
14.....	4.4	23.6	28	26500
21.....	5.9	19.8	28	20000
27.....	4.5	16.2	30	12500
Aug. 3.....	6.6	14.3	32	51000
11.....	5.7	14.4	29	5050
23.....	7.2	8.6	30	15950
31.....	7.4	7.5	30	37000
Sept. 8.....	7.5	6.3	30	65300
22.....	10.2	6.7	19	42200
28.....	10.5	5.3	17	23800
Oct. 12.....	11.2	3.5	20	27400
19.....	10	3.2	19	31000
26.....	12.8	2.8	19	34700
Nov. 3.....	12.3	4	9	18500
23.....	12.8	6.6	12	8700
Dec. 7.....	14.2	4.8	3	10400
14.....	13.2	4.6	2	4900
21.....	15.3	5.3	0	5900

The averages of the results detailed in the preceding tables are as follows:

DESPLAINES AND ILLINOIS RIVERS, BRIDGEPORT TO GRAFTON.

Collecting stations.	Distance from Bridgeport in miles.	Chlorine. ⁵ (Pts. per million).	No. of colonies per cc.	No. of determi- nations.
Bridgeport	0	119.2	1245000	19
Lockport	29	117.4	650000	30
Joliet	33	104.8	486000	28
Morris	57	68.1	439000	26
Ottawa.....	81	58.5	27400	26
La Salle.....	95	46.1	16300	31
Henry.....	123	44.2	11200	29
Averyville.....	159	40.9	3660	30
Wesley City	165	40.9	758000	22
Pekin	175	38.4	492600	29
Havana	199	36.2	16800	26
Beardstown.....	231	29.3	14000	26
Kampsville.....	288	22.9	4800	19
Grafton	318	18.3	10200	28

⁵ The averages of the chlorines here given will not in all cases be found to correspond exactly with the averages of the figures given in the detailed tables; the reason being that occasionally a bacterial sample was discarded or lost and does not appear in the tables, while the chlorine was determined as usual. The averages in this column are based on all the chlorine determinations. The difference is, however, trivial.

PRINCIPAL TRIBUTARIES OF THE ILLINOIS RIVER.

	Chlorine.	No. of colonies per cc.	No. of determinations.
Desplaines River at Lockport	7.9	9180	28
Kankakee River at Wilmington	3.4	5000	28
Fox River at Ottawa	4.99	6510	29
Big Vermilion River at La Salle	61.2	7970	30
Sangamon River at Chandlerville	4.52	5080	21

OTHER COLLECTING STATIONS.

Mississippi River at Grafton	2.8	7600	29
Missouri River at West Alton	15.4	8200	17
St. Louis Tap Water	8.8	2600	19

CROSS SECTIONS OF THE MISSISSIPPI RIVER BELOW THE MOUTH OF THE ILLINOIS RIVER.

	No. of determinations.	E. BANK.		E. CENTRE.		CENTRE.	
		Chlorine.	Bacterial Colonies.	Chlorine.	Bacterial Colonies.	Chlorine.	Bacterial Colonies.
Alton	23	7.6	7900	5.4	7700	3.7	6600
Chain of Rocks	23	5.1	12500	No sample taken.		7.0	12400
Mitchell							
Jefferson Barracks	23	5.6	17700	5.8	13100	6.4	13300

	W. CENTRE.		W. BANK.	
	Chlorine.	Bacterial Colonies.	Chlorine.	Bacterial Colonies.
Alton	2.7	6300	2.7	7700
Chain of Rocks	7.8	12900	8.8	11900
Mitchell				
Jefferson Barracks	7.9	21800	8.2	25200

All the results recorded in the foregoing tables have been obtained upon transported samples of water, and are unquestionably open to criticism upon this score. It will be observed also by reference to the detailed tables, that at most stations the examinations were begun towards the end of the high water period, when the numbers of bacteria were relatively higher than during the prolonged low water period that followed. It proved impossible, however, to arrange for the collection to begin at all the stations at the same time, although every effort was made to this end. At West Alton, owing to local difficulties, the regular collections of Missouri River water were not begun till July 27, so that the average given in the table is considerably lower than would have been the case if high water figures for the Missouri had been obtained. The resulting averages for the Missouri River are hence considerably lower than those for the cross-section at the Chain of Rocks, and a comparison would be misleading.⁶ The averages for all the stations upon the Illinois River are, however, quite strictly comparable throughout.

⁶ The average at the Inlet Tower at the Chain of Rocks for a period corresponding with that covered by the Missouri River analyses is 6900 (Missouri River at W. Alton, 8200).

As has already been stated, we have endeavored to supplement and control the results obtained upon transported samples by numerous examinations made immediately at the point of collection. A series of samples collected at Bridgeport during the summer months and plated directly gave much larger counts than those resulting from the plating of the transported samples. This is undoubtedly due to the destruction of bacterial life in the ice-packed sample (cf. Jordan and Irons, 1899). The correct average from May to August would unquestionably be upwards of 2,000,000. The Lockport samples also, especially those for August, show in a marked degree the diminution due to ice-packing. The Morris samples for July 17, August 21, August 29, September 16 and October 16 also show the effect of transportation; a series of 12 samples collected at different times during these same months and plated *immediately* never afforded numbers so low as those recorded on these dates. On one occasion, for example, three samples of water were plated at Morris immediately after collection (initial temperature of the water 28° C.) and gave respectively 535,000, 412,000 and 329,000 colonies per cc. The bottles were packed in ice by the ordinary method and shipped at once to Chicago, where the samples were plated in the usual routine. The counts obtained after transportation were respectively 54,500, 50,500 and 73,500. If it were necessary, examples of this sort might be multiplied indefinitely.

The diminution of numbers that takes place in ice-packed samples does not, however, result in a stable condition; after a time renewed reproduction sets in, even when the water is kept constantly at a low temperature, and the numbers may rise to a point higher than that originally obtaining. This secondary multiplication occurred not infrequently in the waters from the lower end of the Illinois River and in those from the Mississippi River which had to be transported nearly 400 miles before reaching the laboratory. At Grafton, direct platings from the Illinois and Mississippi Rivers gave almost invariably lower counts than were obtained from the transported samples.

For example:

Grafton, Illinois	River, Oct.	Direct platings	255	345	270
"	"	Shipped "	1200	800	440
"	Mississippi	Direct "		2850	2020
"	"	Shipped "		4500	1500
"	Illinois	Nov. Direct "	225	160	325
"	"	Shipped "	1500	830	580
"	Mississippi	Direct "	1200	850	1150
"	"	Shipped "	2600	5800	540

Multiplication of bacteria in transit was also shown in a marked degree in the samples collected at the Chain of Rocks, where, owing to the fact that the place of collection is difficult of access and a long boat-row is necessary, the packing in ice was unavoidably delayed, and laboratory counts were uniformly higher than those made on samples plated immediately after collection. There is ample evidence, therefore, to support the view that during most of the period covered by these analyses the recorded averages range lower than the true figures as regards the collecting stations near Chicago, and are higher than should actually be the case as regards the more distant points. The apparent difference between the number of colonies found in the Illinois River at Averyville and Grafton, for instance, may be explained in this way; the real difference is inconsiderable.

Early in the investigation the importance of following so far as practicable the changes taking place in one and the same body of water was recognized, but the pressure of routine work rendered such studies few in number. The most important of these were carried out between Morris and Ottawa, where laboratory experience had shown us that a change took place which might be properly denominated as purification. Several series of observations were made upon this stretch of river, but as they all led to the same result only the two most important will be here described.¹

The first of these was carried out on October 7 upon a stretch of the Illinois River just below Morris. The day was bright and sunny, the temperature of the air being 7° C. at six o'clock in the morning and reaching 20.5° by midday. A slight breeze ruffled the surface of the water in the middle of the day, but was at no time strong. The river was very low (5 feet) and the current exceedingly sluggish. The upper cross-sections were taken at a point just above the Mazon River, the lower about three-fourths of a mile below the mouth of the Waupecan Creek. (Neither of these streams was contributing any water to the Illinois at this date.) This stretch of river is almost exactly three miles in length. The rate of flow between the two points was determined by weighted floats and by the use of fluorescein solution and was found to be very close to one-half mile per hour. Four series of cross-sections at hourly intervals were taken at the upper station (A), and these were followed by a similar series at the lower station (B), be-

¹ I am greatly indebted to my chief assistant, Mr. E. E. Irons, for aid in the planning and conducting of these somewhat arduous observations, and I am glad to acknowledge that their accuracy and completeness are largely due to the signal zeal and ability with which he devoted himself to this work.

ginning six hours later. Platings were made within the hour. The samples designated as from the "right" and "left" banks respectively were taken midway between the shore and the centre of the stream. At A the river was about 150 yards wide, at B about 125.

The figures given for the number of colonies are the averages of counts of two separate platings.

UPPER STATION (A).				
Hour.	No. colonies per cc.	Turbidity (Hazen's scale*).	Temp. Water, °C.	Chlorine (pts. per million).
6.15 A. M.				
Right bank.....	500000	.16	13	
Centre	378000	.12	13	
Left bank	42000	.075	13	
7.15 A. M.				
Right bank.....	368000	.17	13	
Centre	344000	.125	13	
Left bank.....	35000	.0775	13	
8.15 A. M.				
Right bank.....	752000	.16	13.5	91
Centre	364000	.11	13	69
Left bank.....	30000	.0675	13	45
9.15 A. M.				
Right bank.....	554000	.16	14	
Centre	472000	.11	14	
Left bank.....	79000	.075	14	

LOWER STATION (B)—(Three miles below (A).				
Hour.	No. colonies per cc.	Turbidity (Hazen's scale).	Temp. Water, °C.	Chlorine (pts. per million).
12.15 P. M.				
Right bank.....	480000	.13	16	
Centre	327000	.15	16	
Left bank	87000	.05	15.5	
1.15 P. M.				
Right Bank.....	281000	.1475	16	
Centre	102000	.09	16	
Left bank.....	19000	.042	16	
2.15 P. M.				
Right bank.....	400000	.13	17	87
Centre	249000	.09	16	72
Left bank.....	22000	.045	16	52
3.15 P. M.				
Right bank.....	412000	.135	17	82
Centre	416000	.12	16	78
Left bank.....	11000	.0433	17	53

* Hazen (1899).

The averages are as follows:

Distance.	No. colonies per cc.			No. of hourly analyses.
	Rt. bk.	Centre.	Lft. bk.	
Upper station A	543700	389700	46500	4
Lower station B (after 6 hours) 3 miles	393250	273500	34750	4
Percentage decrease.	27.6	29.8	25.3	

The chlorine determinations show that the mixing of the Kankakee water and the Desplaines is very incomplete both at the upper station (9.7 miles below the junction of the rivers) and at the lower, and this is entirely confirmed by the bacterial cross-sections at the two points. Between A and B a great bacterial diminution occurs, and this in almost equal degree along the seriously polluted right bank and along the comparatively uncontaminated left bank.

A second series of observations was carried out in a similar fashion upon a longer stretch of river. The distance from the regular collecting station at Morris to the regular collecting station at Ottawa (see map) is about 24 miles, and the rate of flow between the points averaged one-half mile per hour at the time our observations were made. A point midway between Morris and Ottawa was selected (Seneca) and a three-day series of observation was planned. The sun was wholly obscured by clouds during these three days, but no rain fell. The results are given as before in tabular form.⁹

⁹ In connection with this series may be given the averages of the regular chemical determinations for the period between October 23 and November 20.

Station.	No. of determinations.	Residue on evaporation.			Oxygen consumed.		
		Total.	Dissolved.	Suspended.	Total.	By dissolved matter.	By suspended matter.
Morris..	5	398	380	18	10.7	8.4	2.3
Ottawa .	5	356	353.5	2.5	7.5	7.3	.2

Free ammonia.	Nitrogen as			Nitrites.	Nitrates.
	Albuminoid ammonia.				
	Total.	Dissolved.	Suspended.		
7.98	.860	.478	.382	.028	.340
6.5	.364	.315	.049	.332	.648

The Bacterial Self-Purification of Streams

UPPER STATION (Morris).

Hour.	No. colonies per cc.	Turbidity (Hazen's scale).	Temp. water, °C.	Chlorine (pts. per million).
7.15 A. M., Nov. 9.				
Right bank.....	433000	.153	7	
Centre.....	337000	.13	7	
Left bank.....	30000	.046	7	
11.30 A. M., Nov. 9.				
Right bank.....	177000	.17	7.25	67.5
Centre.....	145000	.16	7.25	47.5
Left bank.....	7000	.02	7.25	8.
2.00 P. M., Nov. 9.				
Right bank.....	174000	.15	8	
Centre.....	131000	.135	8	
Left bank.....	49000	.05	8	

MIDDLE STATION (Seneca).

Hour.	No. colonies per cc.	Turbidity (Hazen's scale).	Temp. water, °C.	Chlorine (pts. per million).
9 A. M., Nov. 10.				
Right bank.....	134000	less than .09, more than .07	9.5	51
Centre.....	47000	(Turbidity readings could not	9.5	44
Left bank.....	23000	be taken accurately at this	9.5	35
		point and at Ottawa owing		
		to presence of water weeds.)		
1.30 P. M., Nov. 10.				
Right bank.....	67000		11	
Centre.....	52000		11	
Left bank.....	52000		11	

LOWER STATION (Ottawa).

Hour.	No. colonies per cc.	Turbidity (Hazen's scale).	Temp. water, °C.	Chlorine (pts. per million).
10 A. M., Nov. 11.				
Right bank.....	11000	less than .04	9.5	49
Centre.....	10500		9.5	46
Left bank.....	8900		9.5	43
1 P. M., Nov. 11.				
Right bank.....	12000		9.5	
Centre.....	11000		9.5	
Left bank.....	18000		9.5	

The averages are as follows:

	Distance from Morris.	No. of colonies per cc.			No. of hourly analyses.
		Rt. bk.	Centre.	Lft. bk.	
Upper Station (Morris)	261000	204000	29000	3
Middle Station (Seneca)...	{ 12 miles. 24 hours.	100000	49000	35000	2
Lower Station (Ottawa) ..	{ 24 miles. 48 hours.	11500	10700	13500	2

During this flow of 24 miles, therefore, the Illinois River became nearly free from the great mass of sewage bacteria with which it was originally laden. In fact, the bacterial content of the Illinois at Ottawa was not greatly in excess of that of the local tributary streams."

PROBABLE CAUSE OF THE DISAPPEARANCE OF SEWAGE BACTERIA.

Among the chief causes that have usually been adduced as the essential factors in the purification of streams are dilution, sedimentation, the effect of agitation and aëration, the germicidal influence of sunlight and the activity of the plankton.

Mechanical Agitation and Aëration.—There is no evidence that such mechanical agitation as occurs in a stream so sluggish as the Illinois is injurious to bacterial life in any way. Indeed, the experimental evidence derived from the exposure of bacteria to a moderate degree of agitation (Meltzer, 1894) points to the opposite conclusion; it is true that cell-division is sometimes favored by gentle shaking. Aëration of the water to the extent that takes place in a slowly moving river can likewise be at once dismissed as having no direct untoward action upon the kinds of bacteria appearing in the ordinary plate count, whatever may be its effect upon the strict anaërobes or upon the chemical constituents.

Dilution.—Whenever a polluted stream is diluted with purer water from underground sources or from tributaries the immediate effect must be to diminish the number of objectionable bacteria in a given quantity of the water. This influence of dilution in enhancing the purity of water in a stream is sometimes referred to as not being a "true" purification, although it is difficult to understand just what is gained by such a distinction. If a water contain one hundred typhoid bacilli to the litre and be then diluted to twenty times its bulk with pure water, each litre will then contain only five typhoid germs and, apart from any influence the dilution may have upon the life of the germs, a purification of the water will have occurred to just the same extent as if 95 per cent of the typhoid bacteria had perished, and the danger from drinking a small quantity of such a water would be

¹⁰ The number of colonies found in the water of the Fox River on November 11 was 6850 (average), a number not much lower than that found in the Illinois (11900).

diminished in exactly the same proportion. In the case of the Illinois River between Morris and Ottawa, however, dilution played no appreciable part in bringing about the decrease of bacteria observed during October and November. This is clearly shown by the chlorine determinations. The remarkable fact that the difference between the right and left halves of the river can be traced from the union of the Kankakee and Desplaines to Ottawa, thirty-three miles below, shows the tardiness with which diffusion occurs. A similar failure of the waters of the Illinois and Mississippi to commingle appears from the chlorine determinations at Alton, twenty miles below the mouth of the Illinois.

Action of Sunlight.—As regards the action of sunlight in the particular instance studied by us, nothing very definite can be stated, but such incidental evidences as we gathered do not warrant us in attaching great importance to this factor. In the first place the occurrence of a striking diminution in the number of bacteria, whether the sun shone brightly as in the first Morris series or was entirely obscured, as in the Morris-Seneca-Ottawa observations, forbade the assumption that the main factor in the bacterial disappearance was the germicidal action of the sun's rays. Tributary streams, moreover, like the Fox and Kankakee, showed as high a bacterial content during the months when the sun was powerful as during those in which the sun's influence was most feeble (cf. June and November). The first Morris series affords an interesting opportunity to compare two bodies of water flowing side by side under generally uniform conditions, but into one of which sunlight penetrates much farther than into the other. Reference to the table of turbidities will show that whereas in the left half of the river a platinum wire 0.04 of an inch in diameter could be seen at a depth of 14 inches, in the right half it was not visible more than 6 or 7 inches below the surface. In spite of this material difference in the depth to which light could penetrate, the rate of bacterial decrease was substantially the same on the two sides of the river (p. 307). The main body of the water, if even moderately high turbidity prevails, must be virtually unaffected by the sun's rays.

In this connection it may be stated also that vertical sections have been taken at many points in the course of the investigation without

ever revealing any definite vertical distribution of the bacteria. At Morris, for instance, on November 9, the following averages were obtained:

	{	8 inches below surface.....	177000
Morris, Right bank		2½ feet below surface.....	188000
		4 feet below surface.....	149000
and at			
Ottawa, Midstream	{	6 inches below surface.....	10500
		3 feet below surface.....	8900

I am not, however, prepared to affirm that the sun's rays are always and entirely without effect upon the bacteria in river water, although they certainly play an insignificant part in the case of turbid waters, and cannot be held in any degree responsible for the bacterial decrease that took place between Morris and Ottawa on November 9-11.

Influence of Plankton.—The influence of the plankton is perhaps yet more problematical than that of the sunlight. There are at least two ways possible in which the plankton might exert an influence upon the bacterial population—either by consuming the food-supply of the latter or by devouring the bacteria themselves. I have been unable to satisfy myself, however, that in the Morris-Ottawa stretch of river the plankton is active in either of these particulars. In the first place, the albuminous substances that serve as food for bacteria cannot be as advantageously attacked by the plankton as by the bacteria, and the presence of a few score of diatoms (chiefly *Asterionella*, *Tabellaria* and *Synedra*) is hardly likely to affect materially the main sources of bacterial food-supply. In the second place, while it is possible that some bacteria may fall a prey to the river infusoria, there is no evidence that this can account for any large part of the bacterial diminution, and there is ample evidence from laboratory experiments to show that a bacterial decrease always follows on the heels of a bacterial multiplication in sewage or polluted waters in which there is no plankton.

Moreover, although no extended systematic observations of the plankton have been made, repeated examinations of the water at Morris by the Sedgwick-Rafter method have shown a conspicuous lack of abundant plankton life at this point, and I am hence unable to assign great importance to this cause as a factor in the observed purification.

Sedimentation.—In sedimentation a more potent factor is undoubtedly at work than any yet mentioned. It would be difficult to devise more nearly ideal conditions for sedimentation than those that exist in the Illinois River. In the lower 225 miles of its course there is a fall of only about 30 feet, and owing to the presence of several dams this natural condition of semi-stagnation is still further accentuated by the formation of a series of sluggish pools. “During flood stages the valley is a great lake of say 700 square miles, into which flood waters from above and from tributaries are precipitated and from the lower end of which they run out more at leisure in reduced and equalized volume.” Under these circumstances the settling out of food substances, the entanglement of bacteria in slowly subsiding particles and possibly the slow sinking of the bacteria themselves, all have the fullest play and must all work to diminish the number of suspended bacteria. There can be no doubt that the various influences summed up by the term sedimentation are sufficiently powerful to obviate the necessity for summoning another cause. It is noteworthy that all the instances recorded in the literature where a marked bacterial purification has been observed are precisely those where the conditions have been most favorable for sedimentation.

Exhaustion of Food-supply.—There is one aspect of the subject, however, that demands a little further consideration. This is the limitation placed upon bacterial life by the exhaustion of the food-supply. I am inclined to think that usually insufficient weight is ascribed to this factor. Reference to the chemical analyses at Morris and Ottawa (p.307) will show that a great decrease takes place between these two stations in the “albuminoid ammonia” of suspended matter (from .382 to .049) and in the “oxygen consumed” by suspended matter (from 2.3 to .2). This obviously represents a great removal of bacterial food from the water, and there is no need to assume that this removal is wholly or even in large part due to the process of sedimentation. The destruction of minute floating masses of albuminous substances by bacteria is perfectly competent to account for this decrease in organic substance. It is possible that the larger and more flocculent particles slowly subside and are followed by swarms of bacteria which complete the disintegration on the bed of the stream,

though the vertical distribution of bacteria shows no evidence of this (p. 311). The smaller particles are doubtless consumed while still in suspension. I have been struck by nothing in the course of the investigation so much as by the absence of extensive deposits of the foul black mud popularly supposed to accumulate on the bottom of sewage-polluted rivers. The bed of the Illinois between Morris and Ottawa is singularly free from any deep deposit of organic matter, although the current is very sluggish and sewage in increasing quantities has been poured into the river for thirty-five years. The solid organic matter in the sewage, therefore, is destroyed either while still in suspension or shortly after deposit. The natural effect of this shrinking of the food-supply is to cause a diminution of the bacterial population dependent upon it. That the destruction of large quantities of solid albuminous substances may occur simply through bacterial agency has indeed been conclusively shown in the so-called septic tank method of sewage disposal, and no one can fail to be struck by the general resemblance of the conditions here described to those prevailing in the septic tank.¹¹ The death of bacteria under these circumstances always follows close upon the heels of their enormous multiplication, and whether this be due to starvation or to poisoning by the products of their own activity need not here be discussed. It is sufficient to recognize the fact that the decomposition of large quantities of albuminous substance is first accompanied by great bacterial reproduction and that this is invariably followed by a season of speedy and extreme mortality of the bacteria. In the causes connected with the insufficiency or unsuitability of the food-supply, is to be found, I believe, the main reason for the bacterial self-purification of streams.

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¹¹ The Exeter experiments have shown for instance that the quantity of solid matter arrested in a septic tank might amount in one year to 24.6 tons, and of this only 5.5 tons remained as sludge; 19.1 tons of solid matter disappeared through bacterial activity.

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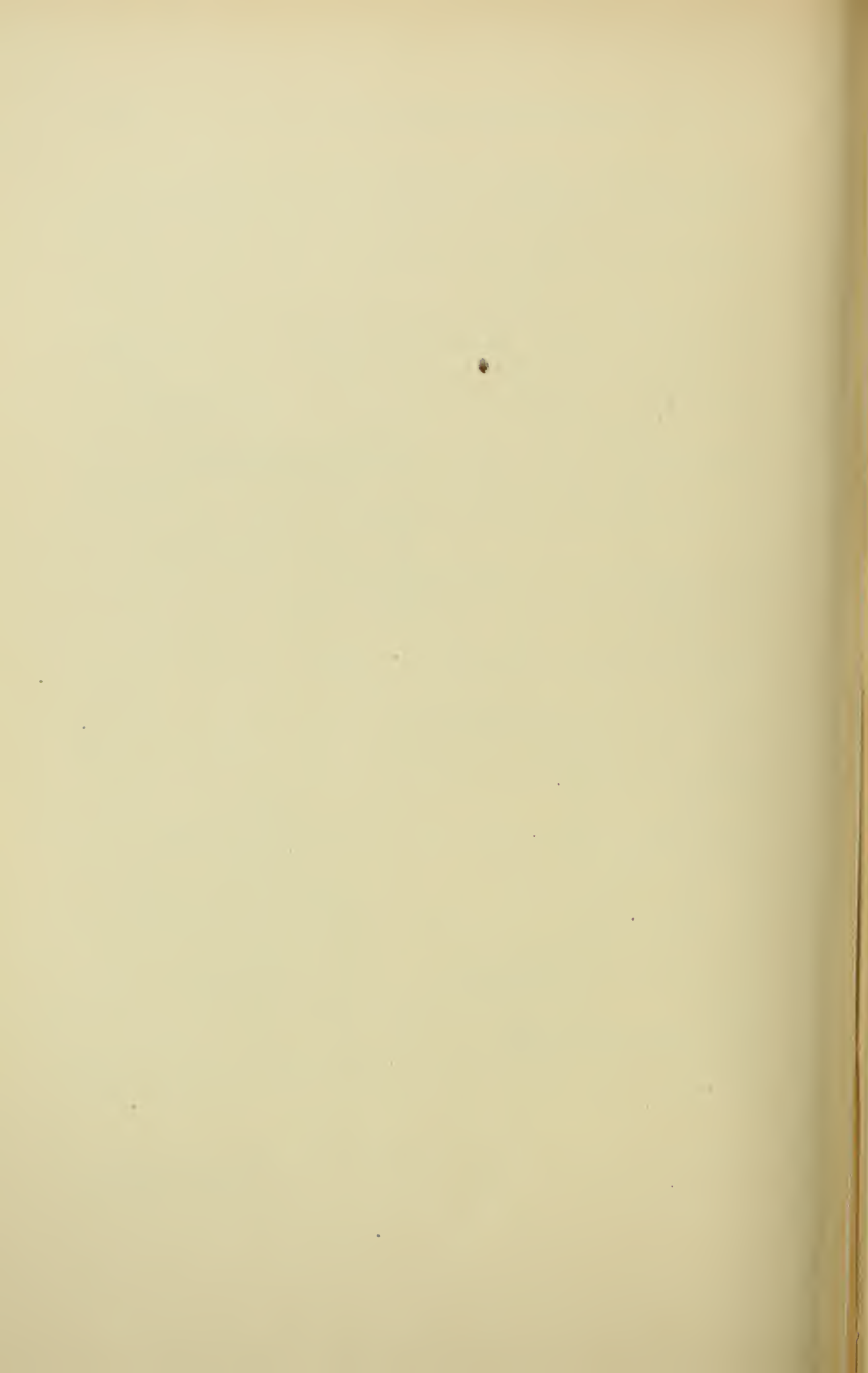
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DESCRIPTION OF PLATE XX.

Map of the Illinois River and its principal tributaries, showing points of collection of samples of water.





ON THE TOXICITY OF NORMAL URINE.

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The purpose of this article is to present the results of some experiments undertaken at the Ohio State University to determine, first, the degree of toxicity possessed by normal urine, and, secondly, the nature of the substance or substances lending toxic properties to this fluid. The importance of this line of work was pointed out by Prof. Bleile, and the investigations were carried on under his supervision.

Before giving the results of these experiments, however, it may be stated, as a matter of history, that poisonous substances were long ago believed by some to exist in normal urine. There were many, on the other hand, who denied the presence of such toxic bodies in this excretion, and, as a consequence, experimental evidence was sought to support each side of the question. Investigations along this line were instigated, in the first place, by the discussion, in the early part of this century, of the cause or causes of uræmia. The first work of any importance was done about that time by Vauquelin and others, who made actual demonstrations of the toxicity of normal urine by intravenous injections. Frerichs, however, claimed that death resulting from such procedures was due to suspended solid elements in the urine and to poisonous ammonium carbonate formed in it by fermentation. Voit was the first to point out that potassium salts, on account of their toxicity, could play an important part in uræmia, and Feltz and Ritter, and Astaschewsky concluded that these salts were the chief toxic bodies in normal urine. Among those who opposed this view were such workers as Schiffer, Pouchet and Bouchard, who satisfied themselves that the toxicity was due largely to the presence of organic compounds of an alkaloidal nature.

Lépine, Guérin, Griffiths, Feltz and others performed experiments

showing that these alkaloidal bodies were increased in certain morbid states, as in measles, diphtheria, pneumonia, cholera, etc. Griffiths claims to have isolated toxins from the urine of pleurisy, influenza, cancer, and epilepsy. Prof. Bleile found that the urine was decidedly more toxic after epileptic attacks, but the alkaloid which Griffiths claims to have isolated from such urine was not found, although his methods were closely followed. Observations on the urine of pathological conditions have lately been very numerous, but satisfactory experimental elucidation of the degree and cause of the toxicity of normal urine is wanting.¹

A complete account of the methods which yielded the results to be stated presently is omitted from this article, as those methods have been published elsewhere. It will suffice to outline the work merely, and to give the more important results.

Urine was collected, usually to the amount of 4 litres, from healthy adults who were not, and never had been, users of tobacco. It was then extracted by Brieger's method,² which after various trials was

¹ The following are among the works which may be consulted for a consideration of the subject of the toxicity of the urine, and for references to the authors cited and the other rather voluminous literature:

C. Bouchard. *Leçons sur les auto-intoxications dans les maladies*, Paris, 1887, also *Lectures on auto-intoxication in disease*. Translated by Thomas Oliver. Philadelphia, 1894.

Neubauer and Vogel (Huppert). *Analyse des Harns*, Wiesbaden, 1898.

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Hymans van den Bergh. Ueber die Giftigkeit des Harns. *Zeitschr. f. klin. Med.*, 1898, xxxv, p. 53.

A. Gottheiner. Die Lehre von den Harngiften in pathologischen Zuständen. *Monatsb. üb. d. Gesamtleist. d. Krankh. d. Harn- u. Sexual-Apparates*, 1897, ii, pp. 649; 713.

A. Beck. Ueber die Giftwirkung des Harnes. *Pflüger's Archiv*, 1898, lxxi, p. 560.

W. P. Herringham. An account of some experiments upon the toxicity of normal urine. *Trans. Path. Soc.*, London, 1899, i, p. 293.

Forchheimer and Stewart. On the toxicity of the urine. *Am. Journ. Med. Sc.*, 1899, cxviii, p. 297.

² Brieger's method (Ueber Ptomaine and Weitere Untersuch. üb. Ptomaine, Berlin, 1885) is described in Vaughan and Novy's *Ptomaines, Leucomains, Toxins and Antitoxins*, Philadelphia and New York, 1896, as well as in other works treating of the same subject. The method, as followed by the writer, was essentially as follows:

Four litres of urine were treated with lead acetate (sugar of lead) until no precip-

found to be the best for extraction of normal urine. The product thus obtained was then injected in a concentrated watery solution subcutaneously into white mice in doses varying from three to seven minims. The following are in brief some of the effects of these injections:

1. Mouse injected with 5 minims of extract of 4 L. normal urine. Somnolence came on in 10 minutes. In 45 minutes severe spasmodic movements appeared. At one time the spasm was general and very violent. These symptoms decreased gradually and recovery took place in about 24 hours.

Seven minims of the same material killed a mouse in 10 minutes, the animal dying in violent convulsions.

2. Seven minims of an extract from another urine killed a mouse in 16 minutes. Respiration became very irregular, the mouse dying in clonic convulsions.

3. Six and one-half minims of another extract caused spasms in the legs. Sometimes the legs would all jerk together, or would be jerked separately, first the front legs together, then the hind legs together. Respiration was irregular and difficult. There was exophthalmus. The symptoms had all disappeared in three hours.

4. Two litres of urine gave two separate masses after removal of the mercury with H_2S , the one soluble in alcohol and the other in water. The alcohol was evaporated from the one part and the mass extracted several times with more alcohol, which was finally evaporated and the residue taken up with water, the slight residue remaining being filtered off. Six minims of this produced in 20 minutes deep somnolence and dilatation of the external arterioles and capillaries. No other symptoms.

itate was formed. The precipitate was then filtered off and the filtrate concentrated carefully to a syrup. This syrup was then extracted with 96% alcohol and the filtered extract was treated with an alcoholic solution of the lead acetate. Any precipitate that followed was filtered off, and the filtrate again concentrated and extracted with 96% alcohol. This alcoholic extract was then concentrated again and taken up with water, the lead in the watery solution being removed by hydrogen sulphide. After filtering off the precipitate of PbS the filtrate was acidified with hydrochloric acid and once more concentrated to a syrup and extracted with alcohol. To this extract a saturated alcoholic solution of mercuric chloride was added till no further precipitate was formed. This precipitate was dissolved in a large quantity of hot water and the mercury was removed by hydrogen sulphide. The water was evaporated and the residue taken up finally in alcohol, which was evaporated when material in solution was to be injected.

Six minims of the original mass soluble in water produced the opposite condition in the external vessels. In 20 minutes the respiration was very weak. In one hour the reflexes were much heightened. These conditions lasted about 6 hours. Mouse recovered.

5. Six minims of extract of 4 L. of urine caused drowsiness only.

6. Four and one-half minims of extract from 4 L. of another sample caused twitching in $2\frac{1}{2}$ minutes. This developed into severe clonic spasms which occurred every few minutes. Respiration was very irregular and finally dyspnoea became pronounced. The reflexes were greatly heightened in 21 minutes, a snap of the finger causing spasm. The exophthalmus was extreme. In 25 minutes the reflexes were abolished. He died in clonic convulsions 26 minutes after injection. The action of this material was much like that of strychnine.

It is apparent, as might be expected, that these urines varied in their toxicity. There was likewise variation in the symptoms. The nervous system was affected most strongly in nearly every case. Respiration was often very irregular and labored. In one case a disturbance of respiration was the only symptom observed. Sometimes the arterioles of the extremities were dilated; at other times they were not. The condition of the heart and pulse was not observed, but it is reasonable to suppose that they were disturbed.

We particularly desired to learn whether these extracts contained any mineral matter. To determine this, in each case a quantity of the extract equal to that injected was evaporated to dryness. Usually a large residue remained. This residue, however, could be easily destroyed by heat, practically no ash remaining after incineration with a Bunsen flame. There was, therefore, no mineral matter in these extracts. Tests for xanthin compounds, it may be stated, gave also negative results.

Evidently we obtained from normal urine a substance, or substances, of an organic nature, possessing marked toxic properties. The effects described above could have been due neither to potassium salts nor to any of the organic bodies usually included in the list of compounds contained in normal urine. No attempt was made to isolate the substance, or substances, that produced the results presented, the intention being to undertake that work at the earliest opportunity.

THE SUPERFICIAL GLANDS OF THE ŒSOPHAGUS.

BY ALBION WALTER HEWLETT.

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PLATE XXI.

The glands of the Œsophagus are of two varieties. Of these the submucous glands have long been recognized. F. A. Schmidt¹ in 1805 described them and there have been many descriptions since then. They are typical mucous glands situated in the submucous coat of the Œsophagus (Plate XXI, Fig. 2). Their ducts pierce the muscularis and frequently show cystic dilatations before emptying by their comparatively narrow mouths. The ducts are lined for some distance from their orifices by a stratified epithelium of cuboidal cells. They frequently pass through lymphatic nodules or lie close to them. The secreting cells and the contents of the ducts stain deeply with stains for mucin.

The other variety of glands is totally distinct from these submucous glands, and as they are best characterized by their situation superficial to the muscularis mucosæ, I shall speak of them as the superficial glands of the Œsophagus (*glandulæ œsophageæ superficiales*). They occur mainly in two localities in the Œsophagus: (1) in areas in its upper portion, and (2) at the transition of stomach and Œsophagus. These latter, the so-called "Cardiadrüsen," extend but a short way toward the stomach, being soon replaced by the typical fundus glands. Toward the Œsophagus they may extend a short distance beneath the stratified epithelium or occur in little groups just above the gastro-œsophageal junction. They have been grouped by some with the gastric glands. The areas of superficial glands which

¹ *De mammalium œsophago atque ventriculo.* Inaug.-Diss., Halle, 1805.

are found in the upper œsophagus will be considered especially in this paper.²

Rüdinger³ described these glands in 1879 as tubulo-acinous glands of the œsophagus lying above the muscularis mucosæ. According to his description they are situated in the lateral wall of the upper part of the œsophagus but are not bilateral. He describes them as consisting of three portions: (1) narrow peripheral tubules lined by pyramidal cells; (2) large cavities into which the tubules empty; (3) the ducts to these cavities which lead to the surface. The cavities and ducts are lined by high cylindrical cells. W. Krause⁴ probably had Rüdinger's work in mind when, in 1879, he classified the œsophageal glands as (1) the common, isolated glands in the submucosa, (2) the smaller glands in the mucous membrane of the lower œsophagus, and (3) a tubular variety of glands at the upper end of the œsophagus. In 1887, Lauteschläger,⁵ after examination of several œsophagi, was unable to confirm Rüdinger's observations.

With these exceptions, Rüdinger's work had passed unnoticed and these glands had been overlooked or misinterpreted, until J. Schaffer,⁶ in 1897, again called attention to them. He had his attention directed to an area $6\frac{1}{2}$ mm. by 4 mm. in the œsophagus of a girl eleven years of age. This proved to be made up of glands superficial to the muscularis mucosæ. He found similar though smaller areas in other œsophagi, but he was unable to find the glands in one œsophagus, from the upper part of which serial sections were made of a piece $1\frac{1}{2}$ centimetres long. He found the glands to be bilateral and located between the levels of the cricoid and the fifth tracheal cartilage. He describes the glands as consisting of a number of twisted and branched tubules of varying diameters

² Schaffer calls these glands "die obere Cardiadrüsen," but as the glands in the fundus of the stomach have been designated by some English writers as "the cardiac glands," I have, in order to avoid the suggestion of identifying these œsophageal glands in structure with the latter, preferred the term "superficial glands of the œsophagus" to Schaffer's designation.

³ Beiträge zur Morphologie des Gaumensegels und des Verdauungsapparates, pp. 27-31. Stuttgart, 1879.

⁴ Handbuch der menschlichen Anatomie, ii, p. 445. Hannover, 1879.

⁵ Beiträge zur Kenntniss der Halseingeweide des Menschen. Inaug.-Diss., Würzburg, 1887.

⁶ Ueber die Drüsen der menschlichen Speiseröhre. *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1897, cvi, p. 175. Beiträge zur Histologie menschlicher Organe. *Ibid.*, cvi, p. 403. Epithel und Drüsen der Speiseröhre. *Wien. klin. Wochenschr.*, 1898, xi, p. 533.

lined by low cylindrical or cuboidal cells. Among these cylindrical cells he found parietal cells in varying numbers in each of his specimens. These tubules empty either directly or by means of wide spaces into the duct. The duct may be dilated, but its opening is always narrow and it always comes to the surface at the top of a papilla. When several such ducts have a common place of emptying, the stratified epithelium is pushed aside and the area is covered by columnar epithelium. Schaffer showed that these glands are morphologically identical with the glands found at the transition of the œsophagus and stomach (Cardiadrüsen).

On the same date that Schaffer presented his first article, Eberth⁷ described an area in the œsophagus which he interpreted as misplaced gastric epithelium. It presented a round, reddish and well circumscribed surface about the size of a five-Pfennig piece at about the middle of the œsophagus. On microscopic examination it was seen that the stratified epithelium ceased abruptly, being replaced by a great number of tubular "mucous" glands analogous to those in the stomach. Eberth did not find similar areas in other œsophagi. Although this area was at a lower level than is common for the superficial glands there seems no doubt but that it was such a glandular area.

Oppel,⁸ in a review of Schaffer's work, speaks of the interesting comparison between the superficial glands of the upper œsophagus and the glands at the transition of the œsophagus and stomach. He deems it important to have a confirmation of Schaffer's work, and especially of the occurrence of parietal cells in the œsophagus. Hildebrand⁹ in 1898 reported a single case of the "occurrence of gastric glands in the œsophagus," which he regards as identical with those described by Schaffer and by Eberth. In his case there were paired areas of glands in the upper œsophagus and these contained parietal cells. In the last edition of Quain's Anatomy¹⁰ it is stated that a few of the smallest of the œsophageal glands are situated in the substance of the mucous membrane. Such are undoubtedly superficial glands. Böhm and v. Davidoff¹¹ describe the œsophageal glands as emptying at the apices of the papillæ, which is true only for the superficial glands and not for the

⁷ Verirrtes Magen-Epithel in der Speiseröhre. *Fortschr. d. Med.*, 1897, xv, p. 261.

⁸ *Lehrb. d. vergleichenden microscopischen Anatomie d. Wirbelthiere*, ii, p. 153. *Jena*, 1897.

⁹ Ueber das Vorkommen von Magendrüsen im Oesophagus. *Münch. med. Wochenschr.*, 1898, xlv, p. 1057.

¹⁰ Quain's Anatomy, edited by E. A. Schäfer and G. D. Thane, Vol. iii, Pt. iv, p. 66 *London*, 1896.

¹¹ *Lehrbuch der Histologie des Menschen*, pp. 170-171, *Wiesbaden*, 1895.

more common submucous glands. Their representation of an oesophageal gland¹² also appears to me to be a superficial gland and not a submucous gland.

In 1899, without previous knowledge of Rüdinger's or Schaffer's publications, the attention of Dr. Ophüls, Professor of Pathology and Bacteriology at Cooper Medical College, San Francisco, was called to these superficial oesophageal glands, by finding two corresponding "oval defects" in the mucous membrane of the oesophagus of a patient who died of pneumonia. These defects were upon its lateral walls, were symmetrical, and were opposite the upper tracheal cartilages, $5\frac{1}{2}$ cm. below the opening of the larynx (Plate XXI, Fig. 1). The long axis of each, measuring 2 cm., was parallel to the long axis of the oesophagus; the width of each was 0.5 cm. The surface was somewhat lower than that of the surrounding mucous membrane and was smooth, glistening and dark red in color, so that these areas looked not unlike ulcers, although on account of their symmetry and regularity of outline this idea was dismissed and a congenital misplacement was suspected. Sections showed these areas to consist of tubular glands superficial to the muscularis mucosæ.

In ten consecutive autopsies, similar but smaller glandular areas were recognized in five cases macroscopically, and these findings were confirmed in each instance by microscopic examination. The size of the areas in these five cases varied from 3 mm. to 8 mm. in the longest diameter. They were circular or more or less oval, and when oval the long axis was parallel to the long axis of the oesophagus. The location in each case fell within the limits as given by Schaffer, namely, on the lateral wall of the oesophagus from the level of the cricoid to that of the fifth tracheal cartilage. They were noted to be bilateral in two cases. One specimen which was at first supposed to contain only a single area was demonstrated upon microscopic examination to present a corresponding smaller area on the other side. This shows how easily a few of these glands may be overlooked. The size of an area therefore may vary greatly: from one invisible to the

¹² Op. cit., Fig. 121.

unaided eye to such a large area as that in our first specimen. There may be only a few glands present, separated from one another by areas of connective tissue and smooth muscle, their ducts opening at separate places through the squamous epithelium. In such cases the squamous epithelium over the glands is of diminished thickness and the papillæ are either small or absent. Such an area appears in the gross as a small nodular bulging in the mucous membrane, usually somewhat larger than the elevations produced by the submucous glands. In other cases, the glands may be so closely packed as to simulate the gastric glands in appearance, although strands of connective tissue are still seen dividing the tubules into groups. In such

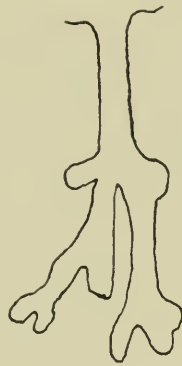


FIG. A.—Diagram of a simple form of superficial gland. Drawn from a reconstruction. $\times 70$.

a case, the stratified squamous epithelium ends abruptly at the glandular areas, the Malpighian layer bending at almost a right angle and becoming continuous with the single layer of columnar cells covering the irregular surface between the mouths of the ducts (Plate XXI, Fig. 2). Such an area looks to the naked eye not unlike an ulcer with well defined edges. It presents a low, red, glistening surface that readily attracts notice on careful examination of the œsophagus.

The glands are of the branched tubular type. They are remarkable for their many windings as well as for their cyst-like spaces. It is rare to see a gland cut longitudinally for any distance on account of the irregular course which it pursues. The complexity of the glands and the number of branches varies greatly. Fig. A drawn from a reconstruction is a schematic representation of one of the simplest

of these glands if its branches were laid in one plane. It is seen to consist of a duct and several acini. The duct (Fig. B, d) is lined by

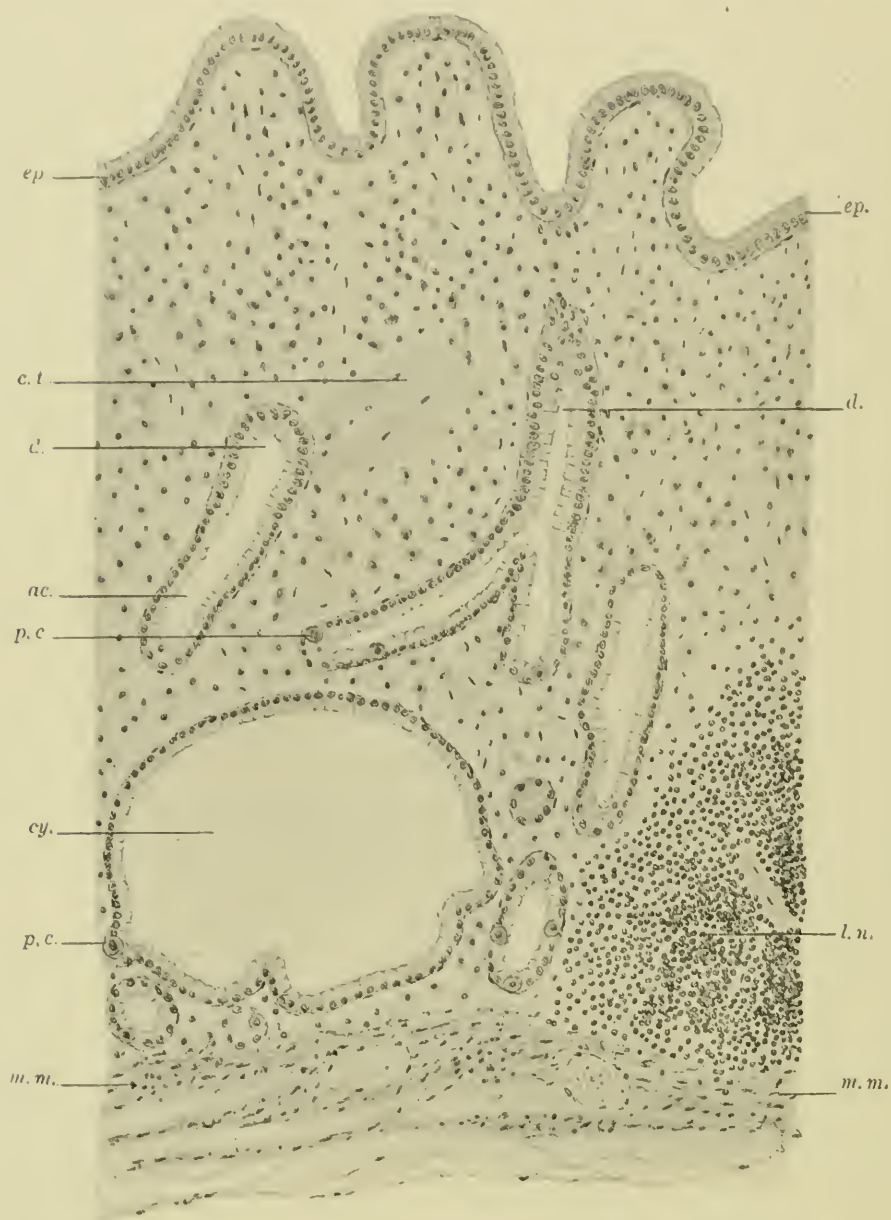


FIG. B.—Drawing of a section of the superficial gland area, $\times 130$. *ep.* columnar epithelium of the surface, *d.* duct, *ac.* acinus, *cy.* cystic dilatation of an acinus, *p. c.* parietal cells, *c. t.* strands of dense connective tissue, *m. m.* muscularis mucosæ, *l. n.* lymphatic nodule.

high columnar cells two to four times as high as they are broad. The nuclei are oval with their long axes parallel to the long axes of the cells. They are situated all at about the same level, a short distance

from the base of the cell. A few ducts contain what appear to be goblet cells among the other cells. Where the gland begins to branch, the character of the epithelium changes. The cells become shorter and more conical. The nuclei become spherical or oval with the axis at right angles to the axis of the cell or even flattened transversely against the base of the cell. Their situation is uniformly nearer the base of the cell than is that of the nuclei of the duct cells (Fig. B, *ac.*, and Plate XXI, Fig. 3). The protoplasm of these acinous cells appears reticulated.

In the acini of many of the glands cells of another type are seen, identical with the parietal cells of the gastric tubules. These are round or oval cells which are usually readily distinguished on account of their property of staining deeply with eosin and picric acid. The nucleus is located at about the centre of the cell (Fig. B, *p. c.*, and Plate XXI, Fig. 3). These cells frequently possess two or three nuclei and occasionally a cell may have four or five. The cell body appears almost homogeneous in some specimens, but in others it is seen to be filled with small refractive granules densely packed together. Clear spaces in the protoplasm, apparently vacuoles, are of frequent occurrence in these cells. It will be seen that in all these particulars they agree in structure with the parietal cells of the stomach. Schaffer states that he found parietal cells in each of his first six specimens. I was not able to find them with such regularity and succeeded in demonstrating these cells in only three of six specimens. They certainly vary greatly in number in the different oesophagi, for in two cases they were present only in small numbers, while in our original specimen they were the prevailing cells in many tubules.

One of the distinguishing features of these glands is the presence of cystic cavities (Fig. B, *cy*). These were present in some of the glands of each specimen examined, but they showed great irregularity in size and shape. Their diameters varied up to 0.7 mm. The epithelium lining these cystic structures is usually like that lining the ducts of the glands. Other cysts, however, and especially those situated deeply in the mucosa, are lined by low columnar cells of the type found in the gland tubules, and, in addition, they may contain parietal cells (Fig. B, *cy.*; *p. c.*). Still other cysts have duct epithelium lining

their superficial portions and glandular epithelium lining their deeper portions. It would appear, therefore, that these cystic structures may be due to dilatations of different parts of the gland. That they are not present in every gland, as Rüdinger thought, is shown by their absence in the gland represented in Fig. A. The portion of the gland which is more commonly dilated is the duct and less frequently it is the secreting tubule, and this accounts for the variability in the cell-lining of the cysts. These dilatations are, as a rule, least frequent in the glands which are richest in parietal cells, and most frequent in glands containing no parietal cells. They are filled usually with a mucoid material containing desquamated cells and cellular debris. These cystic structures are comparable to the dilatations which occur in the ducts of the submucous glands. The function of the latter dilatations has been regarded as that of reservoirs which hold the secretion. As the food passes down the Œsophagus the mucus is pressed out and so serves to lubricate the Œsophagus at a time when it is most needed. The majority of the cystic structures in the superficial glands probably act in a similar manner. Others of these, however, can hardly serve this function. A careful study of serial sections shows that in many cases, especially in the deep-seated cysts, no demonstrable communication exists between the cystic cavity and the surface. Such are, in fact, small retention cysts. The spherical shape of the larger cysts also indicates that the contents are under some considerable tension. In one place a ruptured cyst was seen with extrusion of its contents.

As Rüdinger was the first to point out, the superficial glands lie above the muscularis mucosæ, therein differing essentially from the submucous glands of the Œsophagus. The muscularis mucosæ, as it approaches the gland area, usually becomes diffuse and increases in thickness. Beneath the glands it forms a thin compact layer closely applied to their bases (Fig. B, *m. m.*, and Plate XXI, Fig. 2). Where the glands are some distance apart the muscularis mucosæ may accompany the connective tissue which runs in between them. In such a case the base of the gland is surrounded by muscular fibres and when these contract they probably compress the glands and so aid in expelling their contents.

Schaffer regards the absence of a marked mucin-staining reaction in the superficial glands as an important feature distinguishing them from the submucous glands. There is a mucoid material present in the ducts and cysts, but this does not give a decided mucin-staining reaction. However, in a small number of cells sufficient mucin is present to give a very definite reaction for mucin. These cells are mostly in the lower part of the glands and in my specimens were grouped in two or three little areas, so that probably only a few of the glands contained mucin-producing cells in their acini. Whether these glands are essentially different from those in which the cells gave no mucin-staining reaction was not determined. The mucin is situated in that portion of the cell which is toward the lumen of the gland. Certain cells in the ducts of the glands also take a slight mucin stain. Yet the mucin reaction of the superficial glands as a whole is very slight compared to that of the mucous glands of the submucosa. Even in hæmatöxylin specimens, not too much decolorized, the difference is plainly seen, the submucous glands being of a deep blue color. This is well shown in Plate XXI, Fig. 2.

Lymphatic nodules are present in considerable number in the superficial gland areas. They are located just above the muscularis mucosæ corresponding to their situation in the stomach and intestine (Fig. B, *l. n.*, and Plate XXI, Fig. 2).

The morphological significance of the superficial glands is not clear. Their most interesting feature is the possession of parietal cells, which have been regarded hitherto as the special property of gastric glands. It is not strange that when many parietal cells are present, the glands containing them should be mistaken for a misplacement of gastric glands. As an explanation of their origin in his specimen, Eberth suggests that the squamous epithelium in its growth downward from above circumscribed an island of columnar epithelium which in the growth of the œsophagus became widely separated from its fellow columnar epithelium in the stomach, but like it developed into characteristic gastric glandular tissue. E. Neumann¹³ showed that there

¹³ Die Metaplasie des fötalen Oesophagusepithel. *Fortschr. d. Med.*, 1897, xv, p. 366.

is not a migration of stratified epithelium from above downward but that it develops in situ. Schaffer points out that both the stomach and the œsophagus are derived from the foregut and that while in the œsophagus squamous epithelium is developed, in the stomach the epithelium remains columnar and develops the gastric glands. He considers that a portion of the œsophageal mucous membrane, in failing to change to squamous epithelium, may develop similarly to the gastric mucous membrane and so give rise to gastric glands. In support of his hypothesis Schaffer has described paired areas of simple columnar epithelium in the upper œsophagus of a three-months fœtus. He regards these as an early stage in the development of the superficial glands. Why should this lack of development occur with such frequency in the upper rather than in other portions of the œsophagus? It is well known that the developmental changes in the upper œsophagus are of a more complex nature than in other regions and that this complexity seems in general to favor deviations in development. This may in part account for the gland areas in this region and also for their considerable individual variations.

Schaffer has compared the superficial glands to the œsophageal glands of some of the lower animals. Yet, if we regard the presence of parietal cells as an important feature in these glands, they have no homologue in any described structure in lower animals. The œsophageal glands of birds are above the muscularis mucosæ and in this respect they resemble the superficial glands of man. On the other hand, they are definite mucous glands and show no tendency to occur in such definite paired areas as do the superficial œsophageal glands.

The pathological relations of these glands are of interest mainly on account of their possibilities. It has been suggested that being a place of lessened resistance in the œsophageal wall a large area might gradually give way and so become the starting point for a pulsion diverticulum. The chief support for this interesting speculation comes from the fact that both the pulsion diverticula and the superficial gland areas are situated in the same region of the œsophagus. That the strength of the mucous membrane of the œsophagus is a

very important factor in the resistance to dilatation does not seem probable a priori. Should a large gland area be the point of origin of the diverticulum one would expect to find columnar epithelium in the walls of the diverticulum corresponding to the original area of superficial glands. Of special interest in this regard is König's¹⁴ observation that two of his diverticula contained both cylindrical and flat epithelium. This seems to be the only observation of this kind on record. In a case recently in Dr. Halsted's service in the Johns Hopkins Hospital the wall of the diverticulum was lined uniformly by a pale mucous membrane which proved on section to be covered by stratified epithelium. There was no evidence of the presence of a superficial gland area.

That the glands may be a source of origin for œsophageal cysts seems probable from the fact that they commonly contain numbers of small retention cysts. The largest one in my specimens (0.7 mm.) is just visible to the unaided eye. Ordinarily these small cysts probably cease to grow larger or if they do so they are especially liable to rupture. In a few cases, however, they doubtless continue to enlarge and so give rise to one form of cysts in the œsophageal wall.

The most interesting and important question deals with the possibility of the superficial glands giving rise to carcinomata. Schaffer and Hildebrand both emphasize this possibility. I have collected from the records six cases of adeno-carcinoma of the œsophagus where the locations are mentioned, in order to see if these locations bore any relation to the location of the superficial glands. Of these carcinomata, two¹⁵ were situated somewhat above the middle of the œsophagus, two¹⁶ were somewhat below the middle, and two¹⁷ more

¹⁴ Die Extirpation des Oesophagusdivertikel. *Berl. klin. Wochenschr.*, 1894, xxxi, p. 948.

¹⁵ F. Colle, Beiträge zur Lehre vom primären Oesophaguscarcinom. Inaug.-Diss. Göttingen, 1887.

O. Fischer, Ueber einen Fall von primärem Carcinoma myxomatodes des Oesophagus. *Prager med. Wochenschr.*, 1899, p. 391.

¹⁶ Parmentier, *Arch. gén. de méd.*, 1889, i, p. 470.

Karewsky, Carcinom des Oesophagus. *Deutsche med. Wochenschr.*, 1892, p. 1070.

¹⁷ D. Newman, Malignant Disease of the Throat and Nose. Edinburgh and London, 1892; cited from Rolleston in Clifford Allbutt's System of Medicine, iv, p. 374, New York, 1897.

C. P. White, *Trans. Path. Soc., London*, 1898, xlix, p. 93.

were immediately above the cardiac orifice, yet distinctly separated from the stomach. It will be seen that none of these adenocarcinomata lies within the usual limits for the superficial glands in the upper œsophagus, although the last two lie in the region of the lower superficial glands (Cardiadrüsen). The first four lie in a region where superficial glands are comparatively rare.

That the superficial glands are totally distinct from the submucous glands is easily seen where they are so well developed as in our first specimen. When they are less numerous, however, and are covered by stratified epithelium, recognition is not so easy. The most constant and important differential point is, as we have said, the relation to the muscularis mucosæ, probably all glands completely above this being superficial glands. The superficial glands tend to occur in groups in definite regions of the œsophagus. The mucous glands are generally distributed over the whole œsophagus and lie in the submucosa. The ducts of the submucous glands are lined by stratified cuboidal epithelium; the ducts of the superficial glands by a single layer of columnar cells. Lymph nodules lie about the ducts of the submucous but are beneath the bases of the superficial glands. The superficial glands frequently contain parietal cells, while the submucous glands never do. The mucous glands take a deep mucin stain; the superficial glands take it but slightly. These characteristics show that the superficial œsophageal glands are quite distinct from the submucous glands and, as a rule, are readily recognizable.

In conclusion I desire to thank Dr. Ophüls for his kind permission to report this interesting case of well developed superficial œsophageal glands, as well as for his assistance and the many favors he has shown me during my work in his laboratory.

DESCRIPTION OF PLATE XXI.

Fig. 1.—Photograph of gross specimen which had been preserved in Kaiserling's fluid. The œsophagus is opened along its left side and is turned over to the right. One of the two superficial gland areas is seen as a dark diamond-shaped area on the surface of the œsophagus. The corresponding area of the left side was removed for histological examination.

Fig. 2.—Photomicrograph of a section passing through the edge of the left superficial gland area. Hæmatoxylin and eosin stain. x 17. Above and to the



FIG. 1.

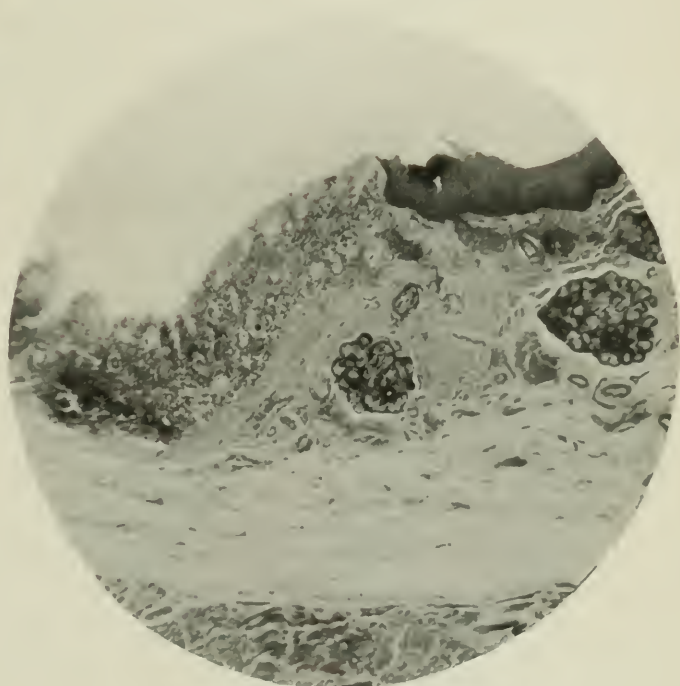


FIG. 2.

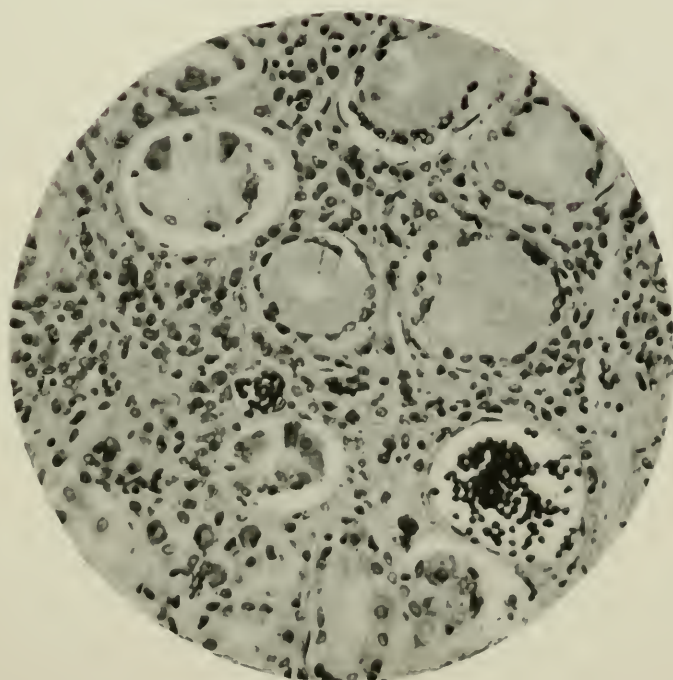
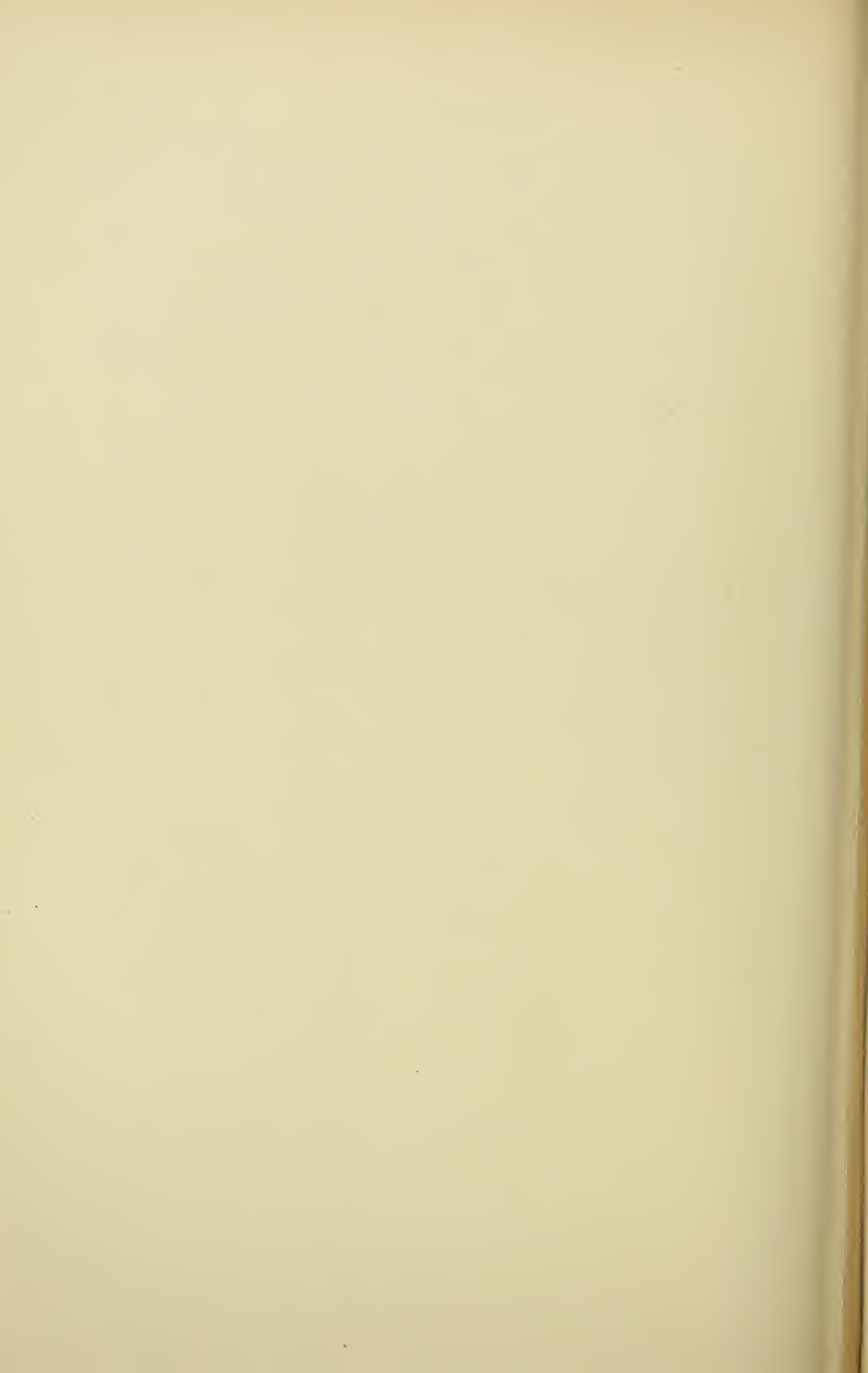


FIG. 3.

right the squamous epithelium is seen, passing toward the left, where it gives place abruptly to the superficial glands. The darkly stained, irregular ovoid masses below the muscularis mucosæ are the submucous glands with the mucus stained by the hæmatoxylin. Their ducts can be seen above them. The muscularis mucosæ is seen superficial to the submucous glands on the right. At the border of the superficial glands it is much thickened. To the left it is very thin and applied closely to the bases of the superficial glands. A lymph nodule is seen on the left just above the muscularis mucosæ. Striated muscle of the upper œsophagus is seen below.

Fig. 3.—Photomicrograph of a section near the bases of the superficial glands, x 220. Stained with Heidenhain's iron hæmatoxylin. Red blood corpuscles are deep black. Near the centre are several acini containing no parietal cells. Acini in the upper left and the lower parts of the figure contain many parietal cells.



FALSE DIVERTICULA OF THE INTESTINE.*

BY MARTIN H. FISCHER.

(From the Pathological Laboratory of Rush Medical College, Chicago.)

PLATES XXII-XXVI.

Diverticula of the intestine may be divided into the true and the false. By a true diverticulum is understood one the walls of which are composed of the entire thickness of the intestine; the false diverticulum, on the other hand, is a hernia of the mucosa and submucosa through the muscular wall (von Rokitsky (1), Leichtenstern (2), Birch-Hirschfeld (3), Orth (34), Hansemann (4), and others). Therefore, while a true diverticulum presents all three coats of the gut, a false diverticulum is formed of mucosa and serosa only, with intervening connective tissue. It is further desirable to differentiate between congenital and acquired diverticula. This distinction, however, does not apply to false diverticula, as, so far as known, all of these are acquired, no case of congenital false diverticulum of the intestine having ever been described.

Edel (5) and others would make synonymous the terms congenital diverticula and true diverticula, likewise the terms acquired diverticula and false diverticula, specifying that the former have all three coats of the intestinal wall, while the latter show only mucosa and serosa. The difficulty in determining definitely in many cases whether the sacculation is congenital or acquired, makes this classification objectionable. A definition based upon morphological rather than developmental conditions, though less desirable, is at least a safe one. Furthermore, Edel's definition does not hold good in all instances, as cases of acquired diverticula possessing all coats of the intestine have repeatedly been reported. Thus Fiedler (6) records such cases. Wallmann (7) has described nine true acquired diverticula of the large intestine. Norman Moore (8) has reported a case of multiple diverticula of the small intestine in a man

* Read before the Chicago Pathological Society, November 10, 1899.

40 years old. The diverticula occurred at the mesenteric attachment of the gut, and were composed of all coats of the intestine. Moore does not state whether he regarded the diverticula as congenital or acquired.

By far the greater number of true intestinal diverticula of congenital origin are the Meckel diverticula, which are formed by a persistence of the omphalomesenteric duct. Other factors may, however, give rise to such diverticula. Grawitz (9) has found a diverticulum of the large intestine whose origin could be traced to fetal malformation. Buchwald with Janicke (10) has described a true diverticulum of congenital origin in the jejunum of a boy 6 years old.

Hansemann (4), Neumann (11), and Nauwerck (12) have each reported a case of true intestinal diverticula with accessory pancreas at their apices. Hansemann's case was of a diverticulum at the upper end of the jejunum of a boy 14 years of age. Neumann found a true diverticulum with a pea-sized accessory pancreas at its apex, a case similar to that of Zenker (13), who found, 54 cm. above the ileocaecal valve, a diverticulum at the apex of which was a cherry-sized pancreas. Nauwerck's diverticulum came from the ileum of a man 43 years old and possessed a separate mesentery. Except Zenker, who interprets his case as one of true Meckel's diverticulum, the observers all attribute the occurrence of the intestinal sacculations to traction brought about by the accessory pancreas. Other instances, in which traction has been believed to be the causative agent, have been described by Birch-Hirschfeld (14). These were caused by connective-tissue bands in the mesentery. In two cases described by Hansemann (4), a tumor produced the traction.

The first description of false diverticula of the intestine is usually attributed to Sömmerring (37) in his translation into German of Matthew Baillie's "*Morbid Anatomy*" (1794), but Voigtel (38) cites previous cases recorded by Schröck, Riolan, Günz, Morgagni and Haller, at least some of which undoubtedly belong to this class. The subject is considered in the text-books on pathological anatomy of Cruveilhier, Rokitsansky (15), Förster (33), Orth (34), and others.

Fleischmann (16) described in 1815 a false diverticulum of the duodenum. Roth (17) in 1872 gave a critical summary of the cases of duodenal diverticula reported up to his time, and described five personal observations. He came to the following conclusions: Diverticula of the duodenum are exclusively of the false variety; usually only one or two, less frequently three or four are present; they do not become larger than a pigeon's egg, and are usually empty; they occur most commonly at the inner, posterior aspect of the pars descendens duodeni at the entrance of the biliary and pancreatic ducts.

Wallmann (7) found in a part of the small intestine, 48 cm. long, thirty-seven diverticula, of which thirty were said to be between the folds of the mesentery. The diverticula were false, the walls being composed only of the mucosa and serosa. Birch-Hirschfeld (3) has described a case in which the ileum through almost its entire length showed many false diverticula along the mesenteric border. Klebs (18) found in the small intestine of an old man twenty diverticula, varying in size from a pea to a walnut, and all situated within the mesentery. Bristowe (19) found just above the ileocaecal valve a solitary diverticulum, the walls of which were formed of mucosa and serosa.

False diverticula of the large intestine have repeatedly been described. Gross (20) described and pictured this condition. Bristowe reported a case of diverticula of the large bowel occurring in a woman 69 years old. The walls of the sacculations were formed of mucosa and serosa only. The diverticula were limited to the site of the appendices epiploicæ, and varied in size from a pin's head to a marble. Alibert found two hundred diverticula in a colon, while other instances of false diverticula of the large intestine have been described by Schröder, Sydney Jones, Astley Cooper, and others.

All writers, with the exception of Förster,* agree that the false diverticula of the small intestine occur almost exclusively on the concave side of the gut, and that they lie between or close to the layers of the mesentery. Their average size varies from that of a pea to that of a pigeon's egg, but Virchow (21) has shown a specimen in which the diverticula attained the size of a hen's egg.

The etiology of false diverticula is still somewhat obscure. Reference has been made to the production of diverticula by traction; this, however, is not generally believed to be the usual cause. It was for a long time held that they were due to increased intra-intestinal pressure of various kinds, as from fæces, or gas. Klebs (22) was the first to point out that a certain relation exists between the blood-vessels and the diverticula. In his case already cited, the small sacs were true, the larger, false diverticula, and all were at the points of entrance of the mesenteric vessels. This condition led Klebs to the suggestion that either through growth of the mesentery in length outstripping

* As suggested by Hansemann, Förster's apparently exceptional position may be the result of a typographical error, as his description otherwise corresponds to that usually accepted.

that of the blood-vessels, or more probably through traction in later life upon the mesentery by the intestine, the funnel-like attachment of the intestinal wall to the entering mesenteric vessels becomes converted into a diverticulum. In support of the latter supposition is the greater frequency of these multiple diverticula in elderly and obese persons.

While Good (23) and Hanau (24), and Hansemann (4) confirm Klebs's observation concerning the relationship of false diverticula of the small intestine to the blood-vessels, they attribute the origin of the diverticula to pulsion rather than to traction, and Hansemann has defined more precisely the vascular relationship. Intestinal diverticula occur in those who at no time have given indications of any notable deposit of fat in the mesentery. In a case reported by Hansemann the patient, who died of pneumonia, was 85 years old and emaciated, having always been thin. About 400 diverticula, varying in size from a millet-seed to a pigeon's egg, were counted, the larger number being in the jejunum and upper part of the ileum. There were some, however, in the duodenum and many in the sigmoid flexure, but none in the vermiform appendix or colon. Those in the small intestine were mostly empty, but some of those in the large intestine contained fæces. Most of the diverticula occurred close to the mesenteric border, but not actually between the layers of the mesentery. Some in the large bowel were on the convex side of the gut. An interesting relation existed between the diverticula situated at the mesenteric border and the blood-vessels; the vessels branched out in the diverticular walls, those beneath the serous surface being arteries, while those visible from the mucous surface were veins. The diverticula had no muscular tunic. The fact that the mucosal hernias were not separated from their corresponding veins led Hansemann to conclude that the false diverticula were formed by the bulging of the mucosa through the muscularis along the sheaths of veins passing from the intestine into the mesentery.

Heschl (25), in 1880, showed that the intestine ruptures under pressure at the points where the diverticula are found. Good, working under Hanau's direction, by filling a loop of intestine with water and

subjecting it to hydraulic pressure, was able to produce trench-like furrows along its mesenteric border before perforation occurred. Hansemann was more successful, as he was able by a similar procedure to produce in the small intestine sacculations which were far more typical than any produced by previous experimenters, and to demonstrate that these bore the same relation to the veins as the false diverticula observed in his patient.

Senility doubtless favors the formation of intestinal diverticula, as this lesion is very rarely found in young persons. Hansemann's attempts to produce diverticula experimentally succeeded when senile intestines were employed, but failed entirely with the intestines of children.

Besides the normal *loci minoris resistentiæ*, other etiological conditions are to be considered. Abnormally great intra-intestinal pressure loses some of its importance as a causative agent when it is remembered how rarely post-mortem examination reveals diverticula of intestines in which such pressure has undoubtedly been present. Chronic constipation may be considered as a predisposing factor, especially in those true diverticula of the colon which represent scarcely more than a dilatation or exaggeration of the normal haustra coli, but here also there is marked discrepancy between the frequency of habitual constipation and the occurrence of diverticula.

Fleischmann pointed out that the muscular fibres of the duodenum are normally separated by the common bile duct, thus furnishing a condition favorable to the formation of a diverticulum in this part of the duodenum. Roth mentioned fatty degeneration of the muscularis as a possible cause. A stenosis, leading to increased intestinal pressure, may be produced by large gall-stones retained within the intestine. Instances of diverticula from this cause are cited by Leichtenstern (2). A retention cyst of the appendix may lead to the production of diverticula in it (Kelynack (26), Ribbert (27)). Finally, attention may be called to the influence, predisposing to diverticular formations, of general sluggishness of the intestinal functions, due to marasmus, phthisis, or carcinoma.

The existence of intestinal diverticula is usually unattended by

clinical symptoms, the presence of the sacculations being discovered only upon post-mortem examination. Sometimes, however, serious pathological processes ensue, as in Sydney Jones's (28) patient, who passed fæces in the urine, in consequence of ulceration of the end of a diverticulum of the sigmoid flexure into the bladder. Alfred Loomis (29) found in the sigmoid flexure and colon descendens of a man 61 years old many highly inflamed diverticula filled with fæces. Although no perforation had occurred, the inflamed diverticula had originated a general peritonitis. Two other cases of peritonitis, associated, however, with perforation, have been described by Fiedler. Klebs (30) has described a case of hernia obturatoria in which a diverticulum had perforated into the hernial sac. One of the favorable terminations of diverticula is, according to Fano (31), obliteration through an increase in the connective-tissue elements. This is quite frequent in congenital but rare in acquired diverticula.

Case I.—This is a museum specimen of a piece of jejunum $7\frac{1}{2}$ cm. long, in the centre of which is a small, oval diverticulum. No other diverticula were found in the intestinal tract. The peritoneum is smooth. The diverticulum is as large as a bean, and lies beneath the peritoneum of the mesentery at one side of the line of mesenteric attachment. Blood-vessels run over its serous covering (Plate XXII, Fig. 1). On the intestinal aspect the valvulæ conniventes are arranged in the normal way. An opening, which admits a small-sized probe, marks the point of communication between the intestinal canal and the diverticulum. This opening is very much smaller in size than the diameter of the sacculæ, and is surrounded by the valvulæ conniventes, three of which dip down into the mouth of the diverticulum, thereby almost closing the opening (Plate XXII, Fig. 2).

Microscopic examination: The diverticular walls are composed of mucosa, submucosa and serosa only. The muscularis stops abruptly at each side of the sac, where it is bunched as though the out-pouching mucous membrane had crowded it aside. The cells of the mucosa are very granular, and their nuclei stain but faintly. The lower portion of the mucosal hernia is highly inflamed. The submucosa is recognizable with difficulty. The muscularis shows faintly stained nuclei, but otherwise no change. The serosa is normal.

Case II.—Emma T., aged 59, a housewife, was admitted to the hospital suffering from pulmonary phthisis and interstitial nephritis. The

autopsy, made by Dr. Hektoen, showed the body of a slender, emaciated woman. The anatomical diagnosis reads: False diverticula of the ileum, chronic pulmonary tuberculosis, chronic nephritis, chronic splenitis, atheroma of the aorta, atrophic liver, general marasmus.

The peritoneum was empty; the omentum adhered to the anterior abdominal wall. The mesentery was of full length. The lower part of the ileum was rather small in calibre; in the first metre and a half were numerous large and small, variously shaped diverticula. The large intestine was normal.

The diverticula are scattered along the mesenteric attachment of the ileum. They vary greatly in size, some being no larger than a split pea, while others are fully half the diameter of the intestine. The larger ones are often lobulated, so that a large diverticulum may present one or more smaller ones upon its surface. The sacculations are covered by peritoneum, which has often been loosened from its intestinal attachment by the out-pouching diverticulum. The larger diverticula show, for the most part, greatly constricted bases, while the smaller ones have broad and expanded ones. The diverticula are not situated exactly between the two mesenteric layers, but usually show a greater bulging on one or the other side of the mesentery (Plate XXII, Fig. 3).

There is an undoubted connection between the blood-vessels and the diverticula. The larger diverticula have the blood-vessels running over their surfaces; the smallest diverticula look as though blood-vessels pierced their apices; at other times, the blood-vessels run over their surfaces (Plate XXII, Fig. 3).

Microscopic examination: All of the diverticula are false, their walls being composed only of mucosa, submucosa, and serosa. The muscularis is separated, the mucosa pushing through it to unite with the serosa and form the saccule (Plate XXIII, Figs. 4 and 5, and Plate XXIV, Fig. 6). The mucosa of the diverticula is faintly stained, granular, and atrophic. In the smaller diverticula it is markedly infiltrated with leucocytes. This inflammation is most marked at the apices of the mucosal hernias, and is most intense in the deeper parts of the mucous membrane. The muscular coat is lacking over the greater part of the diverticulum. On either side of the sac the musculature presents the normal appearance. In some of the specimens the muscular layer shows a sort of club-shaped thickening at the point where the diverticulum begins; sometimes a thin strip of muscular tissue is seen running a part of the way over the wall of the diverticulum at the sides (Plate XXIII, Figs. 4 and 5). When the latter occurs, the muscle thins out gradually until it finally loses itself in the sub-serous connective tissue. The serosa

shows marked increase of connective tissue and often a small amount of round-cell infiltration. In the smaller diverticula a large blood-vessel is usually found at the apex of the saccule. Sometimes more are found, depending in part upon the size of the diverticulum. All these vessels show much thickening of their walls. In no instance have I been able to find intestinal musculature about the blood-vessels of the diverticula. Observations concerning the early development of the diverticula in this case are considered below (p. 343).

Case III.—This specimen of false diverticula of the rectum, sigmoid flexure, and descending colon, was kindly given me by Dr. Le Count.

William P., aged 64, laborer, was admitted to St. Elizabeth's Hospital complaining of pain across the stomach and in the right iliac fossa. The post-mortem examination made by Dr. Le Count gave the following anatomical diagnosis: Diverticula of the rectum, sigmoid flexure, and descending colon; hypertrophy of the heart; general arterio-sclerosis; chronic passive hyperæmia of the liver, spleen, kidneys and lung; œdema of the lower extremities; Meckel's diverticulum; right-sided obliterative pleuritis; slight chronic nephritis; œdema of the brain.

There are adhesions about the splenic flexure of the colon and about the sigmoid flexure. The mucosa of the large intestine is everywhere smooth. In the rectum, sigmoid flexure, and descending colon are found about twenty-five diverticula. The greater number of these are in the rectum and lower portion of the sigmoid. No diverticula are found above the descending colon. They are situated near the mesenteric attachment of the bowel and are from one-half to two centimetres in diameter externally. They differ somewhat in appearance, the smallest appearing as mere digital depressions in the mucosa. The diameter of the orifices is less than that of the saccules with which they communicate. In the larger diverticula labia of mucous membrane surround the openings. The walls of none have perforated, though some are extremely thin. An undoubted relation exists between the diverticula and the blood-vessels. By holding the bowel to the light the vessels can be seen to enter the summit of the diverticula and then, branching, to run over their surfaces (Plate XXIV, Fig. 7).

Microscopic examination: The diverticula are of the false variety. The walls of the larger diverticula are composed of mucosa and serosa only, with intervening connective tissue, but the smaller, beginning diverticula show a muscular wall which has undergone thinning to a greater or less degree. The mucosa of the intestine in general shows no change, but the changes in the mucous membrane of the diverticula are in some places well marked. These consist of a granular disintegra-

tion of the cells associated with loss of nuclei, and an infiltration of the mucosa and submucosa in places with leucocytes. This evidence of inflammation is often most marked in the deeper portions of the diverticula, where, owing to the small diameter of the orifice, communication with the interior of the intestine is difficult. This case was found to be particularly suitable for the study of the early stages of development of diverticula and the results obtained are presented below (pages 344-347).

Case IV.—This example of diverticulum of the appendix was found in the dissecting room of Rush Medical College. I have to thank Dr. Parker for bringing the case to my notice, and Mr. F. M. Wood for details of the pathological findings.

The specimen came from the body of a man about 50 years old, with pulmonary tuberculosis and general arterio-sclerosis. No history could be obtained. The vermiform appendix, bound down by firm adhesions, sprang from the lower and posterior part of the cæcum, running inward, downward and backward so as to lie over the pelvic brim. It is $7\frac{1}{2}$ cm. long and 7 mm. in diameter in its thickest part. It possesses a separate mesentery in which are found blood-vessels and strands of connective tissue. Six centimetres above the tip of the appendix, on the side opposite the mesentery, is a sacculation measuring 12 x 11 mm., with a somewhat constricted base (Plate XXIV, Fig. 8). A few small blood-vessels are seen running over its surface. A little below the site of the saccule, toward the distal extremity, a constriction extends half around the appendix, causing a diminution in its calibre. The appendix, with the diverticulum on the external aspect, is covered by shreds of torn fibrous adhesions. The mouth of the appendix is patulous. By probing, it is found that the lumen is patent only from the mouth to the constriction; beyond this point it is obliterated by connective tissue. The diverticulum and the patulous lumen of the appendix are filled with soft faecal matter in which are found several hard, irregularly shaped, calcareous granules, varying in size from grains of sand to fine gravel. The mucous lining of the patulous lumen is smooth; the wall of the appendix is thickened. The wall of the diverticulum is no thicker than tissue paper; its lining is slightly roughened.

Microscopic examination: The obliterated part of the appendix shows the lumen to be entirely filled up by a loose network of connective tissue; no evidences of mucosa are visible. External to the loose connective tissue is a ring of dense mature connective tissue entirely encircling the appendix. This is evidently the much thickened submucosa. The connective tissue in both these places shows many newly-formed blood-ves-

sels. The muscularis consists of a thick internal circular and a thick external longitudinal coat. No degenerative changes are visible in either layer. The serosa is thickened and presents, at the points corresponding to the torn adhesions, a ragged peritoneal edge. None of the normal lymph follicles are present, and no evidences of an acute inflammatory condition can be found. At the constriction below the diverticulum there is entire loss of the muscular coat, the wall of the appendix here being made up of the connective tissue of the submucosa and the slightly thickened serosa (Plate XXV, Fig. 9). The mucosa over the constriction and extending above and below it, is highly atrophic, remnants only of the crypts being left; at the site of the constriction the mucosa is covered by a thin layer of deeply stained, granular detritus. The submucous connective tissue is greatly thickened and here there are many well-filled, newly-formed blood-vessels; many large empty spaces are present between the strands of connective tissue. No lymph follicles are seen. The muscularis stops abruptly at the lower side of the constriction and more gradually at its upper; the internal layer is thick, the external somewhat thinned. The connective tissue of the serosa is slightly thickened.

The mucosa opposite the large diverticulum is represented by the remains of a few crypts. The enormously thickened submucosa here constitutes the greater part of the wall. It is not inflamed, but many well-filled blood-vessels are seen. Both muscular layers are much thinner than in the sections already described. The serosa is somewhat thickened, so much so at one point that a wedge-like mass of connective tissue has taken the place of the outer muscular coat. The fibrous tissue shows outward pouching.

The wall of the diverticulum is everywhere extremely thin (Plate XXV, Fig. 10). The mucosa of the appendix becomes gradually lost as it merges into the diverticular lining, which is represented by a deeply stained blue line of faecal matter, no actual mucosa being visible. The wall is composed only of submucosa and serosa. The muscularis is gradually lost at the point where the diverticulum begins (Plate XXV, Fig. 10). Many large blood-vessels are found in the wall of the diverticulum. The peritoneal surface shows many torn strands of connective tissue, but no changes of an acute inflammatory nature are seen.

Case I, in being an example of a single diverticulum of the jejunum, contrasts with the cases of jejunal diverticula reported by Virchow (21), Edel (5), and Nicholls (32), which were all of multiple diverti-

cula, such multiplicity being the rule with this type of diverticulum. Roth (17) and others have recorded instances of a single diverticulum of the duodenum, and Bristowe (19) one of a solitary diverticulum of the ileum. The gross and microscopic appearances of the diverticulum in Case I were those characteristic of false diverticula. The intimate relation to the blood-vessels is to be emphasized. The marked inflammation of the mucous lining of the sac is an interesting feature.

Case II is similar to those already cited from Wallmann, Birch-Hirschfeld, Hansemann and Klebs. Hansemann has called attention to the fact that the diverticula in his case did not go directly into the mesentery but lay to one side of it. This same condition has prevailed in both my cases. The tunicary hernia of the jejunum, though covered on all sides by peritoneum, still lay entirely on one side of the mesentery; likewise in the diverticula of the ileum, though some lay just between the mesenteric leaves, the majority showed most prominently on one side of the line of attachment of the mesentery.

My case of diverticula of the ileum is interesting inasmuch as it shows clearly the intimate connection between the blood-vessels and the sacculations. A systematic study of the variously sized diverticula has shown the following points:

The smallest diverticula which could be found—diverticula macroscopically about the size of a split pea—show within the serosa a blood-vessel with much thickened walls. On either side of this vessel may be seen a beginning down-dropping and protrusion of the mucosa. This gives the mucosa a W-shaped appearance, with the blood-vessel contained between the two middle arms (Plate XXIII, Fig. 4). Diverticula somewhat larger show a greater protrusion of the mucosa and an outward movement of the blood-vessel at the apex of the sacculum (Plate XXIII, Fig. 5). Thus the blood-vessel is borne outward by the out-pouching diverticulum. At this stage of development, it seems that one side of the sacculum may grow faster than the other, so that ultimately the blood-vessel is seen to lie at one side of the diverticulum (Plate XXIV, Fig. 6).

This manner of development indicates that the location of a large blood-vessel in a certain place in the muscularis of the intestine pro-

duces here a *locus minoris resistentiæ*; a causative agency of some kind produces an internal pressure which manifests itself by a hernia of the mucosa at the point of least resistance; as no muscular tissue of the intestine is ever found to surround the blood-vessel of the diverticulum, it seems reasonable to suppose that the false diverticulum is formed by a protrusion of the mucosa along the sheaths of the blood-vessels.

Conditions favorable to diverticular formation were present in this case. There was a general marantic condition with chronic pulmonary tuberculosis and chronic nephritis; the intestine, through its entire extent, was thin and flabby; the lower part of the ileum was of small calibre, a condition which, if not due solely to agonal contraction, might at least favor the production of diverticula in the upper part. The inflammatory condition of the mucosa and serosa is also worthy of note.

The detailed study of the process of mucosal hernia through the muscular coat is not so simple as might at first seem to be the case. In the two cases of diverticula of the small intestine above described the thinness of the muscular coat prevented a careful study of the part played by each of the layers, but the thickness of the muscular sheath of the sigmoid and rectum permitted me to study in Case III to better advantage the participation of each in the production of the diverticulum.

As the studies of Hansemann (4) and Graser (35) have shown, the veins draining the intestine pierce the inner muscular layer, run between the two muscular layers, and finally pierce the outer longitudinal layer to make their appearance externally. Graser finds, as previously Hansemann had done, that false diverticula follow the venous sheaths. His view concerning their formation in the sigmoid flexure, to which his studies were limited, is briefly this: a congestion of the veins of the sigmoid brings about dilatation of these vessels, causing thereby a *locus minoris resistentiæ* in the muscular tunic of the intestine; an intra-intestinal pressure such as the presence of gas or fæces furnishes the initiative force required to produce the diverticula at these points of least resistance.

My case (Case III) of diverticula of the rectum and sigmoid flexure corroborates to a great extent the views advanced by Graser. The blood-vessels throughout the specimens examined showed marked congestion, being very large and filled with blood, and there was little difficulty in tracing the connection between the diverticula and the distended blood-vessels. The smallest diverticulum that I was able to find—one that showed macroscopically as a slight digital depression in the mucous membrane—showed the beginning stages of the mucosal hernia. Corresponding to the slight invagination of the mucosa, within the inner layer of the muscular tunic was found a large congested blood-vessel; on either side of this a well-marked thinning of the muscular sheath had already occurred; this thinning was most evident in the inner muscular layer, the outer layer being still intact (Plate XXVI, Fig. 11). A somewhat larger diverticulum showed on histological examination a greater depression of the mucosa with a greater thinning of the inner muscular layer (Plate XXVI, Fig. 12). For some time at least, the outer longitudinal muscular layer may remain intact, being pushed more and more outward by the protruding mucosa (Plate XXVI, Fig. 13). As the diverticulum continues to grow the muscular layer surrounding it becomes very much thinned and finally disappears entirely, so that the wall of the diverticulum is ultimately composed of mucosa, submucosa, and serosa only.

The foregoing description does not, however, apply to all the diverticula. Although the inner, circular coat usually seems to be the one to give way first, it sometimes happens that the outer shows the first break in its continuity. It would seem that in these cases the blood-vessel had occupied the outer layer of the muscular tunic, in this way changing the locus minoris resistentiæ from the inner to the outer layer of the muscularis. Thus such conditions as I have pictured in Fig. 14 (Plate XXVI) come to pass, in which the mucosa and the inner muscular layer rupture through the outer one. In this process the blood-vessel is evidently pushed ahead of the oncoming diverticulum.

Finally, it would seem that under some conditions a break occurs simultaneously in both layers of the muscular sheath. This occurs in those places where several large vessels are situated closely together,

so that the entire muscular wall is so weakened that the mucous membrane breaks through both with comparative ease (Plate XXVI, Fig. 15).

It is now the generally accepted belief that the diverticula follow the paths of the venous sheaths (Plate XXVI, Figs. 16-17); still, though this be in the main correct, I cannot accept it in its entirety. Although the presence of the blood-vessel is the prime, predisposing cause leading to the production of the mucosal hernia, still, once started, the sacculæ follows the path of least resistance. This may or may not be the sheath of the blood-vessel. Instead of following the latter, the mucous membrane may spread between the layers of the muscular tunic (Plate XXVI, Fig. 13), or it may spread through the connective tissue between the muscularis and the serosa in places where it is impossible to establish any connection between the diverticulum and the blood-vessels.

The partially successful effort of nature to limit the enlargement of the diverticula is evidenced by the connective-tissue hyperplasia about them. The thickness of the serosa covering the diverticula is usually almost directly proportional to their size; and there can be no doubt that to this protective attempt of nature is due the infrequency of perforation of diverticula into the peritoneal cavity.

Doubtless an important factor in the causation of the diverticula in Case III was the chronic passive congestion of the viscera due to hypertrophy and dilatation of the heart and arterio-sclerosis. While this factor has not the exclusive importance attached to it by Graser, it is certainly one of the demonstrable predisposing causes of intestinal diverticula. Constipation furnished another condition favorable to the process by increasing the intra-intestinal pressure.

The presence in this case of firm adhesions about the rectum and sigmoid, corresponding to the position of the diverticula, is also of interest. As has been stated, some of the diverticula were highly inflamed. It can readily be understood how easily these pockets, communicating as they do through only small openings with the lumen of the intestine, might harbor faecal matter, the accumulation of which might lead to an inflammatory condition. How difficult the removal

of such material may be, is evident from Fig. 13 (Plate XXVI), which shows a section through one of these highly inflamed diverticula. Graser has pointed out the relation which may exist between diverticula of the lower bowel and chronic pelvic peritonitis. I interpret the adhesions which were formed about the sigmoid flexure in my case as an evidence of such a chronic pelvic peritonitis engendered by the inflammatory condition of the diverticula. Further evidence of such a peritonitis is found in the pain in the iliac fossa of which the patient complained. A few diverticula were found in the region of the beginning descending colon, and here, too, slight adhesions had formed.

Case IV is an example of diverticulum of the vermiform appendix—a condition concerning which but little has been written. Finkelstein (36) has suggested the possibility of the production of a diverticulum from increased intra-appendicular pressure following occlusion of the mouth of the appendix and consequent collection of secretion. Two cases have been recorded. That of Kelynack (26) was of an appendix which became cystic in consequence of obstruction to its opening. Two very distinct diverticula communicating with the lumen of the appendix were found between the folds of its mesentery. No muscular tissue was detected in the walls of the diverticula and hence the conclusion seems justifiable that they were mucosal hernias through the muscularis in consequence of the pressure produced by the stasis of the secretions within the appendix.

In Ribbert's (27) case there was a group of small cysts of varying size, arranged in a grape-like cluster at the mesenteric border of an apple-sized cyst of the appendix and communicating with the latter through larger and smaller openings. The large cyst was the result of occlusion of the mouth of the appendix. The small cysts are interpreted by Ribbert as dilated glandular cysts which had become partly occluded and separated from the mucosa. If this interpretation be correct, these cysts would not be analogous to the ordinary false diverticula of the intestine.

In some respects, more like my case is the one reported by Edel (5), who found in a man 69 years of age a pea-sized false diverticulum, situated on the side opposite the mesentery, 2 cm. from the distal end

of the appendix, which was 8 cm. in length. A small blood-vessel ran over its surface. There were no faecal contents in the appendix or diverticulum, and its lumen was patent. The muscularis was absent over the diverticulum. The mucous membrane was atrophic; the serosa showed many new blood-vessels. By the side of the diverticulum the muscle was replaced by a wedge-shaped mass of connective tissue, which, though of unknown origin, was thought to represent a scar. For the formation of the diverticulum in this case Edel offers the following explanation: the scar produced a slight traction upon the mucosa and, as the result of connective-tissue increase in the serosa, the latter began to bulge out. Assuming that the traction upon the mucosa increased, and that the muscularis finally yielded to the slight but continued pressure of the mucosa, he was led to believe that the production of a false diverticulum thereby resulted.

I am unable to say what caused in my case the mass of adhesions found about the appendix and its diverticulum. A tubercular or syphilitic etiology is possible. There were no acute inflammatory lesions, and in the absence of a history we must leave this point unexplained. If we believe with Ribbert that obliteration of the appendix represents a process of involution and not one of pathological change, then little is to be said concerning the obliteration of the lower part of the appendix in my case. Loss of lymph follicles and replacement with hyperplastic connective tissue are both found, so that all the conditions described by Ribbert as characteristic of this type of obliteration are represented.

The constriction of the appendix on the distal side of the diverticulum is interesting in so far as it presents the typical structure of a false diverticulum without a bulging of the weakened part. The wall of the appendix at the point of the constriction is covered with a mucosa upon which lies a deeply stained granular detritus. Longitudinal serial sections passing from the non-constricted into the constricted portion of the appendicular wall show a progressive thinning of the muscular coat, ending finally in its total loss (Plate XXV, Fig. 9). Under ordinary conditions, we would expect the mucosa to bulge outward as the muscular coat became thinner, still in this case, the oppo-

site has occurred and the serosa has fallen in toward the mucosa. This seems to me to be a natural sequence when we remember that the connective tissue of the submucosa is fully four times as thick as, and hence, firmer and more unyielding than the connective tissue of the serosa. Thus, instead of the formation of a diverticulum, a constriction has resulted.

The cause of the production of the large diverticulum can at best only be surmised. As in Edel's case, it occurred on the side opposite the mesentery, so that its formation as a mucosal hernia along a vascular sheath is extremely unlikely. It may be recalled that the appendix was embedded in firm adhesions; that the diverticulum was filled with faecal matter; and that the wall was composed of thickened serosa only. True intestinal diverticula due to traction produced by an accessory pancreas, or by connective-tissue bands in the mesentery have several times been described. It seems to me not impossible that in the beginning this diverticulum of the appendix was a true one, due to the traction of a connective-tissue band, and that this mural bulging became filled with faecal matter, the accumulation and stagnation of which caused a pressure atrophy of the mucosa and muscularis of the diverticulum. Thus the wall of the diverticulum became weakened and composed of connective tissue only.

As we have no means of learning whether the adhesions antedated the diverticulum, another explanation may also be suggested. It may be that in consequence of the pressure and irritation of faeces accumulated in the appendix the mucosa first and then the muscularis underwent necrosis or simply atrophy. There would thus be produced in the wall of the appendix that which is essential to the formation of a diverticulum in any part of the intestinal tract, namely, a point of lessened resistance, which, under the further influence of an increased intra-intestinal pressure, as from meteorism or constipation, might readily be the starting point of a diverticulum presenting the histological features observed.

The possibility may also be considered that the same process, whether pathological or referable to simple involution, which had caused the constriction, may have affected a limited area in the wall

of the appendix causing a focus of lessened resistance, which yielded to the normal or perhaps increased pressure within the lumen of the appendix, before any considerable compensatory new connective tissue had formed.

I am indebted to Professor Hektoen for much kind assistance in this investigation and in the preparation of this paper.

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DESCRIPTION OF PLATES XXII-XXVI.

PLATE XXII.

Fig. 1.—Case I. Jejunum, showing the relation between the blood-vessels and diverticulum.

Fig. 2.—Case I. Arrangement of the valvulae conniventes of the jejunum about the diverticulum.

Fig. 3.—Case II. Ileum, showing the intimate connection between the diverticula and the blood-vessels.

PLATE XXIII.

Fig. 4.—Case II. Section of a diverticulum in process of formation. The atrophic mucosa is seen dipping down on either side of the blood-vessels. The muscularis is much thinned on the left and entirely absent on the right side of the diverticulum.

Fig. 5.—Case II. A later stage of development of a diverticulum. The muscularis is missing in the wall of the diverticulum. On the right it has been crowded aside by the out-pouching mucosa. Several blood-vessels with much thickened walls are seen in the wall of the diverticulum on the right.

PLATE XXIV.

Fig. 6.—Case II. Section of a mature diverticulum with greatly thickened subserosa. The left side of the diverticulum has evidently grown more rapidly than the right. A large blood-vessel is seen in the right wall of the diverticulum. The mucosa has undergone marked degeneration.

Fig. 7.—Case III. False diverticula of the sigmoid flexure. The blood-vessels are seen running over the surfaces of the diverticula.

Fig. 8.—Case IV. Appendix vermiformis, showing diverticulum, constriction and mesentery. The adhesions are not shown.

PLATE XXV.

Fig. 9.—Case IV. Section through constriction of appendix. The muscularis is wanting in this portion of the wall of the appendix.

Fig. 10.—Case IV. Section through diverticulum. The subserosa and the submucosa of the appendix are seen to merge into a single layer to make the wall of the diverticulum.

PLATE XXVI.

Fig. 11.—Case III. First stage in the formation of a diverticulum. The mucosa is dipping downward toward the dilated blood-vessel in the somewhat thinned internal coat of the muscularis.

Fig. 12.—Case III. A later stage of the same. The muscularis has become still thinner about the blood-vessel.

Fig. 13.—Case III. The mucosa has broken through the internal coat of the muscularis and has pushed the external coat ahead of it. A lateral pouching of the mucosa has taken place between the internal and external layers of the muscular tunic. The subserosa is much thickened, and in it are several blood-vessels.

Fig. 14.—Case III. The mucosa in this instance has pushed the internal muscular coat ahead of it, and with it has broken through the external one. A large blood-vessel is seen at the apex of the diverticulum.

Fig. 15.—Case III. Simultaneous rupture of both muscular coats of the intestine. Attention is called to the great number of large blood-vessels found in and about the muscularis at the site of rupture.

Fig. 16.—Case III. A diverticulum in close relation with a blood-vessel.

Fig. 17.—Case III. A diverticulum, associated with rupture of both muscular coats of the intestine, making its way along the sheath of a blood-vessel.

Addendum.—Since the completion of this paper I have examined two additional cases of false diverticula of the sigmoid and rectum (in one case with also a few diverticula in the descending colon), both being in tuberculous men—40 and 60 years old—with chronic passive congestion from cardiac and vascular disease. There were 25 diverticula in one, and 10 in the other case. The histological conditions were like those in Case III.



FIG. 1.

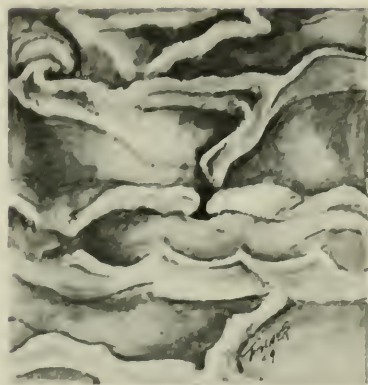


FIG. 2.

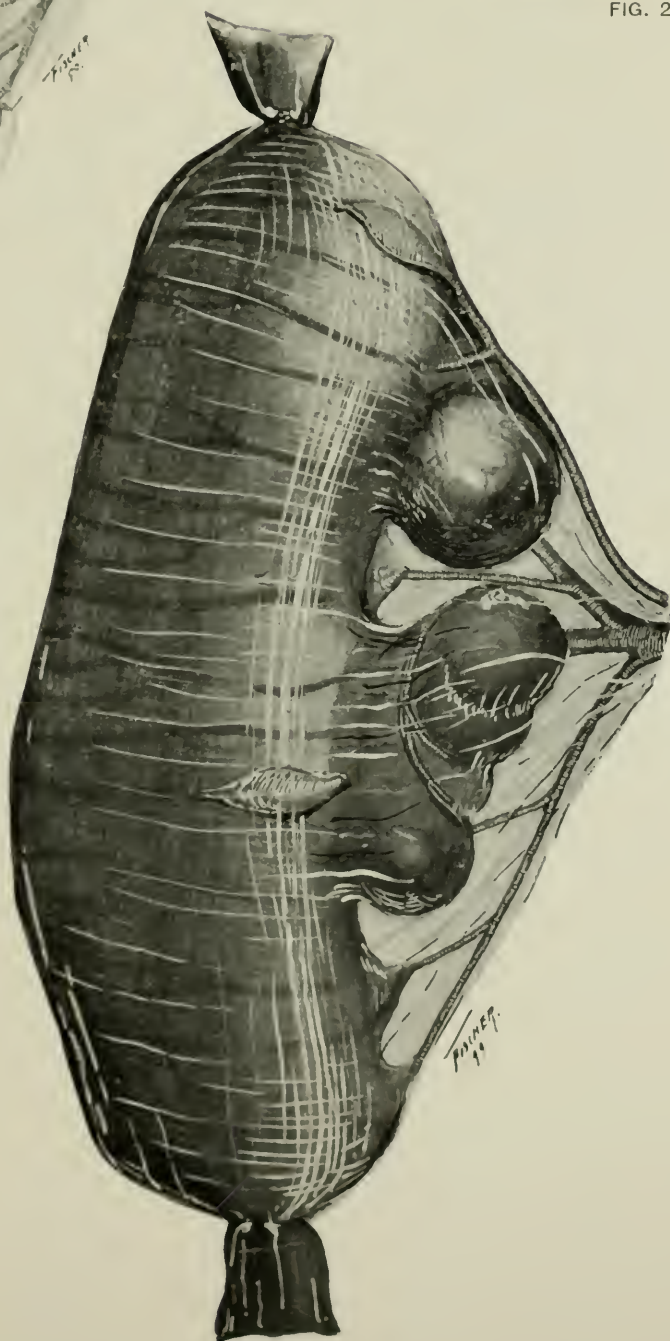


FIG. 3.

35



FIG. 4.



FIG. 5.

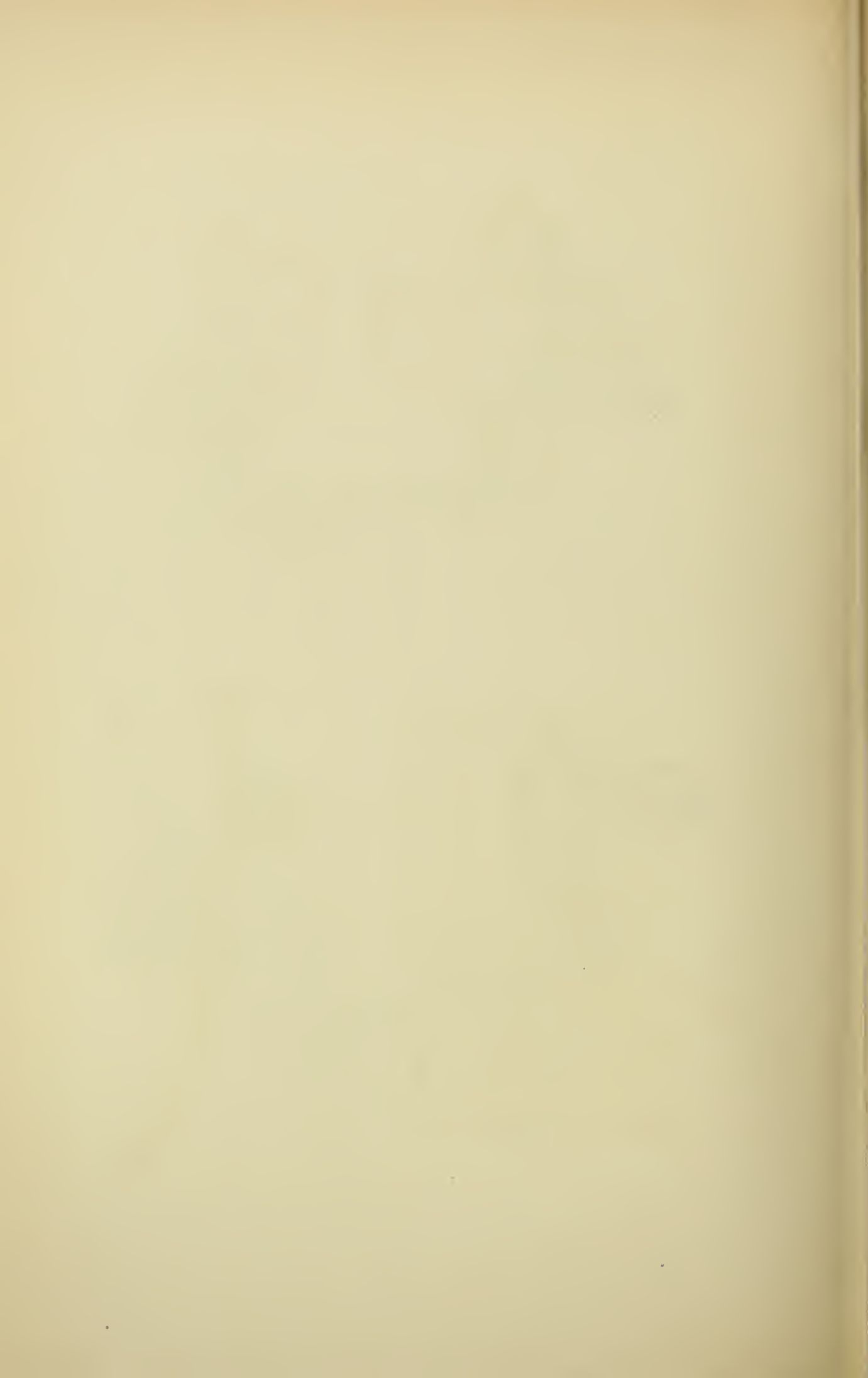




FIG. 6.

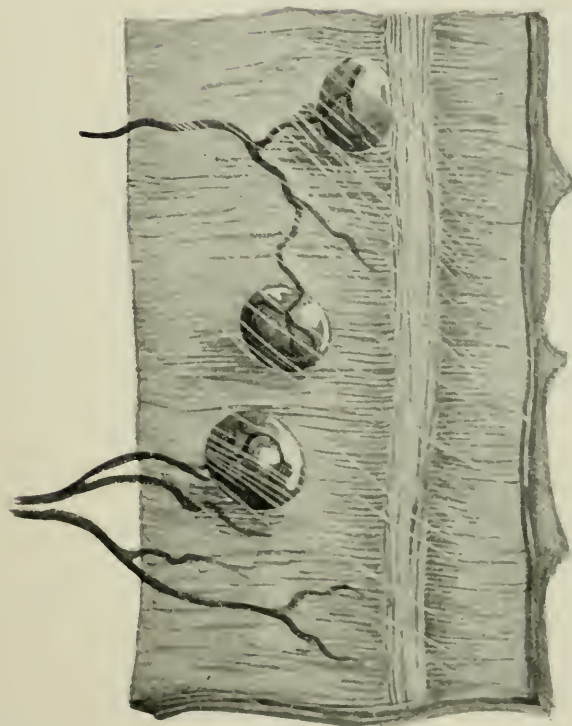


FIG. 7.



FIG. 8.

3524



FIG. 9.

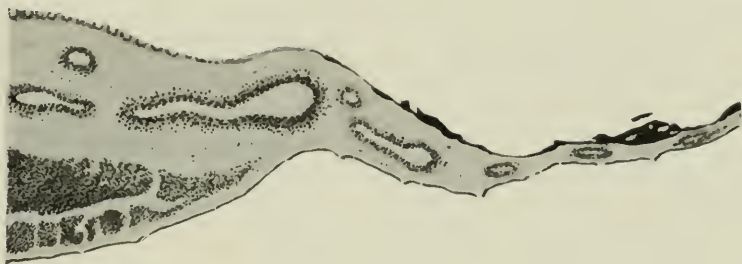


FIG. 10.

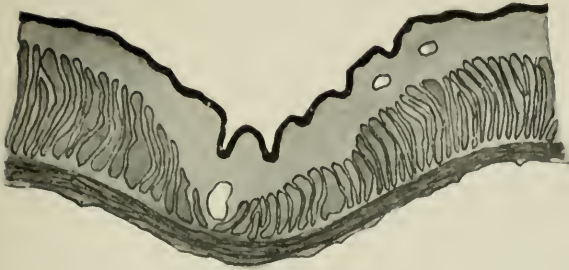


FIG. 11.

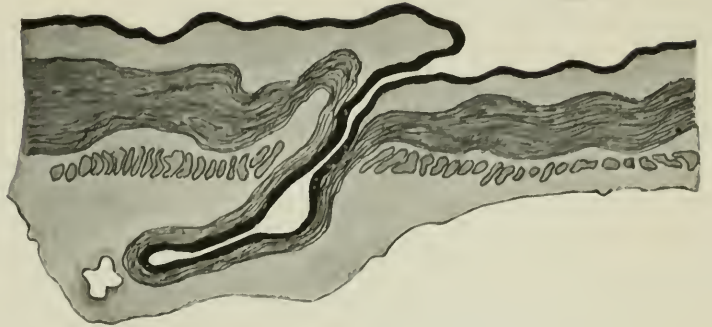


FIG. 14.

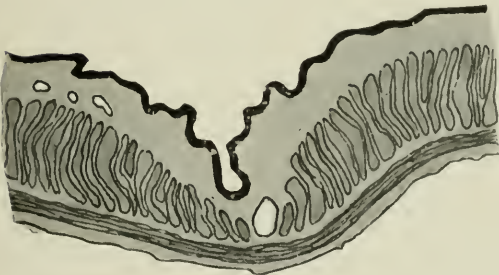


FIG. 12.



FIG. 15.



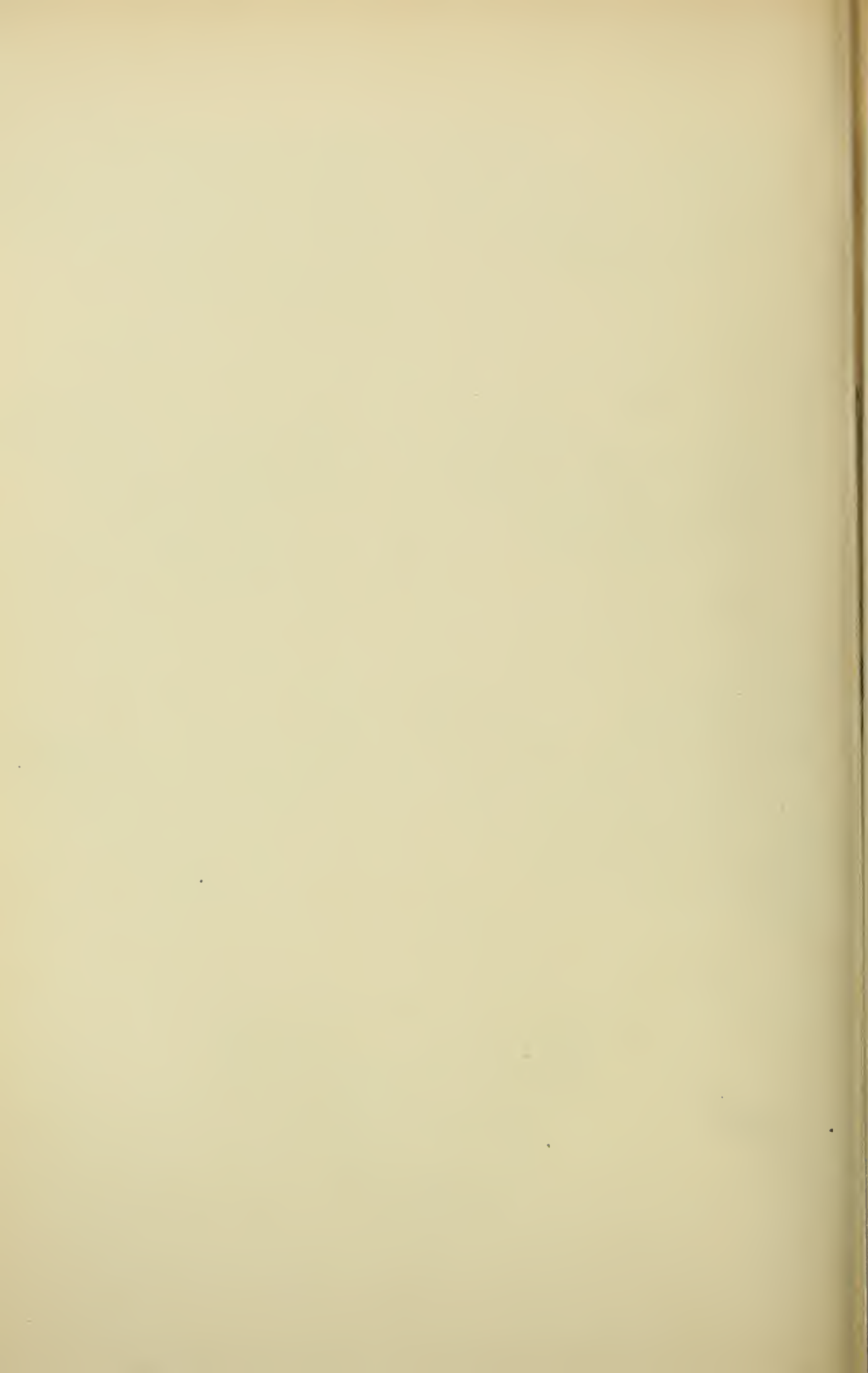
FIG. 13.



FIG. 16.



FIG. 17.



SOME THEORETICAL CONSIDERATIONS UPON THE NATURE OF AGGLUTININS, TOGETHER WITH FURTHER OBSERVATIONS UPON BACILLUS TYPHI ABDOMINALIS, BACILLUS ENTERITIDIS, BACILLUS COLI COMMUNIS, BACILLUS LACTIS AEROGENES, AND SOME OTHER BACILLI OF ALLIED CHARACTER.¹

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(From the Pathological Laboratory of the University of Cambridge, England.)

	CONTENTS.	PAGE
	Introduction	354
I.	Agglutinating sera	354
	Their special action upon certain bacterial races.....	354
	Theoretical considerations upon the constitution of serum of immunised animals.....	359
	Method of preparing sera for agglutination tests	362
II.	Other means of differentiating the groups of bacilli under consideration...	366
	Summary of classification	367
	Division I. Typhoid-like morphology.....	368
	Group A. Type: <i>B. faecalis alcaligenes</i>	368
	Group B. Type: <i>B. typhi abdominalis</i>	368
	Group C. Type: <i>Bacillus "Gwyn"</i> and <i>Bacillus "O"</i>	369
	Group D. Type: <i>B. enteritidis</i>	369
	Division II. Colon-like morphology.....	370
	Group E. Type: " <i>urethra</i> ".....	370
	Group F. Type: " <i>425</i> ".....	370
	Group G. Type: " <i>+ A</i> " and others	370
	Group H. Type: <i>B. coli communis verus</i>	371
	Group J. Type: <i>B. coli communior</i>	371
	Note on non-motile colon bacilli	372
	Division III. <i>B. lactis-aërogenes</i> division.....	373
	Bacilli not to be confused with the foregoing groups.....	374
	Review of some tests ordinarily applied.....	375
	Morphology.....	375
	Cultural tests.....	375
	Litmus milk-whey	378
	Fermentation tests	380
	Methods.....	380
	Factors to be considered in appreciating fermentation tests.....	381
	Precautions in preparation of media and in testing.....	381
	Influence of nature of sugar, etc., employed.....	383
	Influence of amount of sugar, etc.....	383
	Influence of other constituents of the medium.....	384
	Is the sugar altered by a bacillus which does not produce acid or gas?	385
	Preliminary search for other sugars and fermentable substances	385

¹ This article having been written without access to a library, I have taken the liberty of inserting in foot-notes the references to some of the authors cited in the text.—EDITOR.

Owing to my participation in an expedition for the study of certain tropical diseases, it is improbable that I shall be able to continue, or to render so complete as would be desirable, an account of the studies which have been made during the past five years. However, although these studies have been interrupted, the following notes may be of some interest to those working in the same field, especially since many points require further elucidation. The work upon groups of bacilli to be considered was begun in November, 1894, in connexion with the question of serum tests. These studies, together with those upon vibrios, initiated the method of testing by the clumping or agglutinating action of sera of immunised animals upon appropriate organisms.²

I.—AGGLUTINATING SERA.

THEIR SPECIAL ACTION UPON CERTAIN BACTERIAL RACES.

Further experience with the differential actions of sera of treated animals tends to confirm the conclusion that so far as bacteriolytic and

² Since many writers continue to use the name of Widal in connexion with some of these reactions, it may be pointed out that so far as priority of publication is concerned, there can be no doubt that Gruber was the first to publish the fact that human typhoid-fever patients acquire clumping power of their blood serum towards the typhoid bacillus. The whole principle of the test was established from Prof. Gruber's laboratory, and from the historical side the question of the exact period of the illness at which the reactive power is perceptible, is a mere matter of detail. Grünbaum has pointed out that some of the original cases referred to by Gruber at the Wiesbaden Congress in April, 1896, were as early as the 10th or 11th day of the fever. It may also be noted that a Fellow of the Royal Society of London was interested in the paper which I presented to the Royal Society (published in January, 1896) and which, owing to the delays in the printer's hands, was not published in full in the *Journal of Pathology and Bacteriology* until July, 1896; and he made a long abstract of this contribution which he sent to the editor of a French medical periodical. This abstract, I understand, neither was acknowledged nor was it published. Not long afterwards the application of the clumping test to the diagnosis of typhoid fever was published in France, apparently without much reference to Gruber's remarks made in April. I understand that the editor of the above-mentioned periodical was then F. Widal. Anyhow, from the point of view of priority of reference to the matter in print, if any name should be attached to the reaction it should be that of Gruber. I feel impelled to call attention to these points, since, although I personally was concerned with working out the prime foundations of the agglutination test for artificially prepared serum (and Grünbaum in the case of human typhoid fever), yet, so far as I am concerned, the inspiration was derived from my respected teacher Professor Max Gruber, as, indeed, I have already recorded in my paper in the *Journal of Pathology and Bacteriology*, 1896.

agglutinating actions are concerned, the word "specific" is inapplicable. In my previous paper³ it was suggested that the word "*special*" would be a better one to employ. It will be remembered that Prof. Gruber and I found that within the "species" *Vibrio cholerae asiaticae* the serum reactions were not uniform, in that the serum obtained by immunising with one race did not necessarily give more than a trace of reaction in vitro and none whatever in vivo when tested with another race, although it was capable of giving complete clumping and positive Pfeiffer reaction when tested upon the first race. At that time we ascribed this to differences in virulence of the races, for the serum obtained by the use of non-virulent cultures had little or no effect upon the most virulent stocks; on the other hand, the serum obtained by the use of virulent cultures affected both the non-virulent and the virulent stocks of cholera vibrios. Since then, however, I have found that certain less virulent strains of *Bacillus enteritidis* derived from the same source were less affected by a given serum (both in vitro and in vivo) than the more virulent strain. The matter is one which possesses considerable complexity, and we (and Pfeiffer in following us) were probably incorrect in ascribing the difference to a mere difference in virulence.

Further, in discussing the "specific" value of the test, I found that two clearly differentiable vibrios—the "Massowah," with its four flagella (not the "Massaouah" used by Bordet and others in the Institut Pasteur, which reacts as a cholera vibrio with serum, as well as in its cultural characters), and the "phosphorescent Elwers" vibrio, with its single flagellum and other special characters—were, so to speak, *serum-identical*, that is, the serum of either affected the other not only by the clumping test, but also by the Pfeiffer bacteriolytic test and the protection afforded to animals. It seemed from these observations that the serum test, both in the test-tube and in the animal, could not be considered a final criterion for the diagnosis of species; for, first, there was some want of uniformity in the action of serum tests within the "species" cholera vibrio (especially when the serum

³ H. E. Durham, On a special action of the serum of highly immunised animals. *Journal of Pathology and Bacteriology*, 1896, iv, p, 13.

identity of V. "Berolinensis" of Rubner and V. "Versailles" of Sanarelli and V. "Iwánoff" are remembered), and secondly, there was mutual serum identity when apparently perfectly distinct "species" (such as V. "Massowah" and V. "Elwers phosphorescent") were investigated.

Since then I have made a number of agglutination tests upon members of the *B. enteritidis* group. So far as cultural and morphological tests are concerned, I could not certainly diagnose these races from one another, it being understood that comparative observations were made not only at a single time, but now and again in some cases over a period of years and in others of months. Temporary variations do occasionally occur, but these are not constant and, after all, are insignificant in that they do not affect main characteristics. So far as these characters go we have a well-defined group, yet, when tested by serum for agglutination, marked differentiation may be found. Thus the races "Gärtner" and "Morseele" are both strongly affected by "Gärtner" serum, whilst the races "Hatton," "mor-bificans bovis," "psittacosis," "Aertrycke," "Calmpthoult," "Gand," "Sirault," "hog cholera," "typhi murium," "Sheffield" (kindly sent to me by Dr. Robertson), are not very markedly affected by this serum. To some extent the race "Günther" would appear to be intermediate; still it is more markedly affected by the serum of the Hatton type. Taking the reverse view, we find that the serum of "Hatton" has comparatively slight effect upon the races "Gärtner" and "Morseele;" thus a serum which gave good reaction at 1 in 200,000 upon "Hatton," practically gave but a minimal reaction upon "Gärtner" and "Morseele" at 1:2000. It may also be mentioned that Gärtner serum, though efficient against "Gärtner," has practically no protective effect against the living Hatton bacilli; so that here within a group of bacilli, the characters of which can be more closely studied than is the case with the vibrios, the serum test does not give material aid in defining its limits. At the same time the Gärtner type has a distinct tendency to be affected by the serum derived by the use of typhoid bacilli. Here again we find differences between the typhoid bacilli, for instance the serum of the race

.

"Weichselbaum" has much more effect than that of my race "HS," though here it is far less than that produced upon any of the typhoid races (22) with which I have directly compared them. To put the matter in a short way, it might be said in the formula of a proportion sum that "Hatton" is to "Gärtner" as "Gärtner" is to typhoid "Weichselbaum."

Two very interesting cultures from the Pathological Laboratory of the Johns Hopkins University, "Gwyn" and *Bacillus* "O," for which I have to thank Prof. Flexner and Dr. Harvey Cushing respectively, have also yielded evidence that the clumping test has only a limited value in the diagnosis of species. Both of these were obtained from cases clinically resembling typhoid fever.⁴ They are both distinguishable from the typhoid bacilli and also from the enteritidis group in their cultural reactions, but I hardly think that I could distinguish one from the other with certainty, so much do they resemble each other. Both of them fail to give the slightest clumping reaction with typhoid serum (potency 20,000-100,000) or with various enteritidis sera (potency 50,000-500,000); of the latter, "Gärtner," "Hatton," "Günther" and "morbificans bovis" have been tried. I prepared a "Gwyn" serum, active up to 1:20,000 upon "Gwyn," but this has not the slightest effect upon Dr. Cushing's *Bacillus* "O" at 1:100 dilution. Here the serum reaction confirms the notion obtained from cultural tests that these two bacilli are different from the typhoid and the enteritidis bacilli, but from their similarity it might be expected that they would shew some mutual reaction; this, however, is not the case.

One more example may be given, and it is of some value in shewing that distinctly differentiable bacilli of the colon group may simulate one another by their mutual serum reaction. *Bacillus* "W," a member of the *B. coli communis* verus race (vide, p. 371), has no power of fermenting sucrose (cane-sugar), *Bacillus* "G" will readily ferment this sugar; both bacilli have been under my observation for fully four years, and they have retained these characters and can be differ-

⁴ Gwyn, *Bulletin of the Johns Hopkins Hospital*, 1898, ix, p. 54. Cushing, *ibid.*, 1900, xi, p. 156.

entiated from each other by culture with ease. Now, both "W" and "G" are mutually susceptible to the clumping serum test, so much so indeed that one sample of "W" serum acted on a culture of "G" up to about 50,000 dilution, whilst upon "W" itself it fell out about 30,000.

Next it was found that apparently indistinguishable true *B. coli communis* races were not mutually affected by highly potent sera (none of which was less than 50,000 potency). This is a point which was foreshadowed in my earlier paper, and which, indeed, has been worked at by a number of observers with a like result. I think, however, that they have not worked with such highly potent sera, and that they thereby have saved themselves the time and patience which in some respects I have wasted. So far as my experience goes, it is rather unusual to find two otherwise indistinguishable colon bacilli from different sources, which give any mutual serum reaction in test dilutions. I have now tested a good many different cultures during the past four years and have almost always obtained completely negative results, although the sera were highly potent for their own races. In the light of the oft-repeated statements of Prof. Baumgarten, that the serum of rabbits readily agglutinates colon bacilli, I may state clearly that this is not my experience. I rarely test in lower dilutions than 1 in 200, and at this or higher dilutions of rabbit's serum (whether normal or immunised) the results are practically always negative. It is possible that his results may be partly due to the use of cultures in broth containing muscle sugar, which, when mixed with fresh broth or with serum, may give some precipitation of proteid matters and thus carry down the bacilli, giving rise to their apparent agglutination. In order to avoid such fallacies, I use agar cultures rubbed into a suspension with sterile 1 per cent NaCl solution; this is a standard method when a given parallel-sided loop and equal amounts (say about 2 mgrm.) of bacilli per 1 cc. are used. In sedimentation tubes, I find that the reading after 18-20 hours is generally the same as that taken after 48 or 72 hours, there being little or no multiplication of the bacilli.

THEORETICAL CONSIDERATIONS UPON THE CONSTITUTION OF SERUM OF
IMMUNISED ANIMALS.

It should be understood that mutual reactions may be partial or complete, but in speaking of these I do not include reactions which are obtained with test sera below 1:1000. The effects produced upon bacilli by the serum of apparently normal animals in less dilute conditions can only be considered to be of a special nature, when it is *not a constant peculiarity* of the species of animal, and then to avoid fallacies dilutions not less than 1:100 must be employed. Just as we are at present unable to evaluate the position of the antitoxic action of the sera of some "normal" animals, so we cannot yet assign a position to these clumping actions of the sera of normal animals in low dilutions. Personally, I believe that both antitoxic and clumping characters in such sera owe their origin to the presence and absorption of appropriate bacillary products, but it is also possible that mutually reacting substances, *i. e.* bacillary product and serum constituent, are present, the latter of which is not necessarily identical with the true agglutinins. I may instance the precipitating effect of quillaic acid upon peptone solutions, as a case where two organic substances interact, whilst other glucoside constituents of *Quillaia* bark do not have this effect.

But to point my moral more aptly, an illustration may be taken from the precipitating actions of ricin and abrin upon sera. Solutions of the proteoses (albumoses) obtained from the seeds of *Ricinus* and *Abrus* (like the globulins obtainable from the seeds) form a precipitate when a drop of serum is added; the serum of normal rabbits, guinea-pigs, rats, fowls, horses and hedgehogs all produce this effect. But working with rabbits I find that the amount of precipitate obtained by adding 10 cmm. of serum to 1 cc. of a solution of *Ricinus* albumose is far more copious when the serum is taken from an animal which has been immunised with the *Ricinus* albumose than when taken from a normal or an abrin-treated animal. Moreover, the precipitation can be obtained in dilutions of the albumose solution with anti-ricin-albumose at which the normal or anti-abric serum fails to give any precipitate. Only abrin albumose precipitates both kinds of serum

(anti-ricin and normal) in like degree. I may add that the anti-ricin serum has a protective effect, whilst normal serum has none, when the serum and ricin are given in recently made mixtures.

I made some experiments with abrin, which shew that in the act of precipitation by normal serum much of the toxicity of a given solution may be removed. Thus 0.01 cc. of a given abrin solution killed 300-gramme guinea-pigs in about 60 hours, 0.02 cc. in about 48 hours, and 0.05 cc. in about 30-40 hours. To some of the same solution normal rabbit serum was added (0.05 serum to 3 cc. abrin), this was allowed to settle for a few hours and the clear supernatant fluid tested; it was then found that 0.01 cc. of the original solution failed to kill, whilst 0.05 cc. killed only after about 60-70 hours. The nature of this action is not clear and I do not propose to discuss it here. The point is that by dilutions in vitro at any rate a quantitative difference between the "coagulins" can be detected, and the protective action of the special serum is suggestive that there is a qualitative difference also.

It will be noticed that I have been comparing the bacillary agglutination process with the action of precipitating agents, more or less after the theory propounded by Kraus.⁵ I do not think that bacillary agglutination is due purely to an entanglement of the bacilli in coagula formed in the free fluid. The microscopical observation of bacilli mixed with very dilute special sera is most suggestive of some alteration of the surfaces of the bacilli in the direction of increased stickiness. It may be that this surface alteration is due to a precipitation or more or less "nascent" precipitation upon the surfaces of the individual susceptible bacilli; such bacilli as are secreting more of appropriate substances will be more susceptible to the action of the serum and become more profoundly affected. It is always a striking phenomenon that all the bacilli are not equally influenced in a given dilute mixture.

In order to explain the somewhat perplexing partial and mutual reactions of agglutinating sera upon different groups or races of bacteria the following more or less graphic method may be suggested.

⁵ *Wiener klin. Wochenschr.*, 1897, x, p. 736.

I suppose that a given "agglutinin" is not a single substance, but a complex one, the constituent elements of which I will designate by capital letters, whilst the bacillary components which are capable of giving rise to the formation of the agglutinins (when introduced into an animal) may be represented by corresponding small letters. Thus if we take typhoid and enteritidis sera which give some mutual reaction, they will be graphically represented thus:

Elements concerned with agglutinins	B. typhi.	B. enteritidis (Gärtner type).	B. enteritidis (Hatton type).
Serum constitution	A, B, C, D, E	C, D, E, F, G, H	E, F, G, H, J, K
Bacillary constitution	a, b, c, d, e	c, d, e, f, g, h	e, f, g, h, j, k.

When typhoid serum containing (A + B + C + D + E) is added to typhoid culture (= a + b + c + d + e) the maximum effect of clumping is produced where each substance reacts upon the other; when it is added to Gärtner bacilli (= c + d + e + f + g + h) it will only produce an effect when the substances C, D, and E are able to affect a certain proportion of the bacilli or rather their constituents c, d, and e to a sufficient degree; still greater must be the concentration of the typhoid serum to produce an effect upon the bacilli containing only the susceptible substance e.

The matter is further complicated; for instance, it may be supposed that although two typhoid races both consist of a + b + c + d + e, these substances are not present in equal quantities. Thus one typhoid race or individual bacillus may be represented by the formula 20a + 10b + 5c + 2d + 1e, and another by 1a + 2b + 5c + 10d + 20e. When tested by a third serum containing all the constituents in equal quantity (*i. e.* A = B = C = D = E) they may give identical dilution limits, but they will not do so when tested by means of their own sera. Moreover, any given race does not necessarily produce the same quantities of the different constituents at different times, and hence the variations of agglutinability, virulence, etc. Further, I suppose that the bacteriolytic, inhibitory and protective or preventive substances have a similarly complex constitution, the amount of each unit being to some degree independent of the other, though all the substances tend to be grouped together more or less

dependently. These hypothetical considerations appear to be simple and at the same time a fairly satisfactory explanation of intricate phenomena of the various actions of the sera of immunised animals.

METHOD OF PREPARING SERA FOR AGGLUTINATION TESTS.

It seems from numerous experiments that the most satisfactory method of producing clumping sera is to give considerable quantities of killed bacilli by means of intraperitoneal injections. It is possible to get moderate potency by giving sterile filtrates of cultures, as I shewed in my paper⁶ in 1896, a discovery which has been variously claimed by or ascribed to Levy and Bruns or Widal, all of whom, however, described the fact about a year or so later. It is also possible to induce some power (about 1:4000 is the highest I have obtained) by giving killed cultures by the mouth; were evidence wanting that the agglutination is no "reaction of infection" this might be cited as such.

Rabbits are the most satisfactory animals to use, chiefly because a good supply of blood can be obtained from them from time to time. About one-tenth of an agar culture killed at 60°-65° C. is a good dose to begin with for a young rabbit of about 900 grammes (I prefer to begin on young animals) and this is increased gradually according to the gain in weight at intervals of a few days at first and of a few weeks later. The injections should be given on the left side of the abdomen in the inguinal region; if a blunt needle is used and the skin perforated by means of a small cautery there is no risk of wounding the intestine, for this is the region of the small intestine. The sites of the cæcum and stomach should be noted and avoided. In the guinea-pig the right side, about midway between ribs and pelvis, is the proper site for intraperitoneal injections. I have had several rabbits under treatment for periods of 2-3½ years, and they have received very many injections without causing any adhesions of the viscera.

When the dose of bacilli from 3 or 4 agar tubes has been successfully passed, it is economical and convenient to smear the surface of agar which has been allowed to set in Petri dishes. In order to smear the surface rapidly, I have made use of the turn-tables which are used for ringing microscopical specimens with cement; by removing the spring clips and putting 3 small pellets of modelling wax an excellent revolving support is made for the purpose. A quantity of culture is scraped from a gelatine or agar slope and smeared on to a broad platinum spatula

⁶ *Journal of Pathology and Bacteriology*, 1896, iv, p. 13.

made of a piece of foil soldered to a wire and mounted on a holder. The dish is set spinning and the bacteria are rapidly spread.⁷

When the culture has grown the bacteria are scraped up with a loop and emulsified with saline solution and then killed by exposure to a temperature of 60°-65° C. for about half an hour.

It should be noted that broth is not a suitable substance for making the injection. I discarded it at first for reasons of economy, but the recent work upon the precipitins and coagulins, especially that of Dr. Walter Myers,⁸ shews that in animals treated with peptone injections substances capable of precipitating these bodies are produced in the serum. Such a serum, when mixed with a peptone broth culture of bacilli, may cause some apparent agglutination of the bacilli by virtue of an entanglement with the precipitated proteid. A parallel effect is seen in the clumping of mica particles in a mixture of typhoid serum and filtrate of typhoid culture, as in the experiments of Kraus, or by the clumping action on indifferent blood corpuscles, as Myers has shewn.

In the treatment of the animals it is best to give injections increasing up to about four Petri-dish cultures. A series of injections of smaller quantities every day for a week, followed by an interruption and then another series of injections, is not quite so good, I think, for the preparation of highly potent sera. It appears probable that a smart reaction should follow the injection; in fact, for the highest potency the final injections should be near the maximum recoverable dose. Thus two rabbits, which had been under treatment for rather more than two years, and which had received equal doses at the same intervals for the last 12 months, were each given a considerable dose of the same material; one of the rabbits died in about 24 hours and had a clumping potency of about 1:200 at death; the other was very ill for a time but developed a potency of over 1:2,000,000. With this mode of treatment it is easy to obtain sera of a potency of 1:200,000, but it is more difficult to increase the potency up to and above 1:500,000.

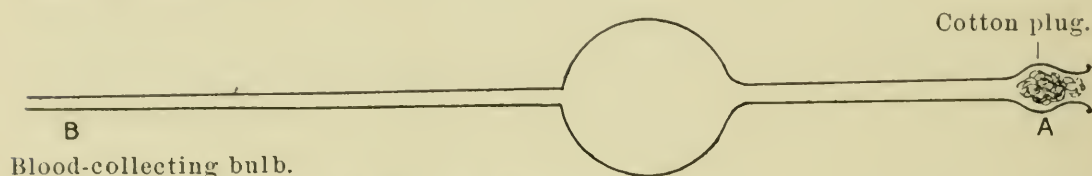
To obtain blood, the ear of the rabbit is shaved; the skin is cleansed with lysol (2 per cent) and soap and thoroughly dried with sterile cotton wool; the basal vein of the ear is compressed, and when the minute vessels are quite turgid, a small cut is made in the marginal vein by means of a sharp lancet. The blood will flow rapidly from quite a small puncture, and, indeed, will often "pump" as if from an artery. It is taken

⁷ The same method is useful for separating impure cultures, the same spatula or a comb-like instrument (made by cutting a number of cuts in a piece of platinum foil) being used to smear successive plates whereby discrete colonies may be obtained.

⁸ On immunity against proteids. *Lancet*, 1900, ii, p. 98.

up by means of sterile bulbs, with suction if necessary, from the pool of blood upon the skin.

Many forms of tube have been tried, but that shown in the diagram seems the most useful apparatus. The blood is then blown out into sterile test tubes, which are inclined to the maximum limit allowed by the tube in order to get a thin layer of blood clot. The tubes should not be touched until the blood has thoroughly well clotted, otherwise some blood-corpuscles will be liberated and become mixed with the serum. After an hour or so the tubes are stood up vertically. By these means some 10-15 cc. of blood and a corresponding quantity of serum may be obtained with ease from a rabbit without any operation beyond a mere prick in the vein; no pain is induced and no anæsthetic is required, the animal sitting perfectly still and shewing no signs of discomfort. The process may be repeated from day to day if desired and a considerable amount of perfectly *sterile* blood-corpuscle-free serum easily obtained.⁹



A. Mouth-piece with cotton plug.

B. Collecting capillary about 2 mm. diameter.

When it is desired to bleed the animal to death, I do so from the carotid artery, under an anæsthetic. In order to obtain a maximum quantity of serum, it is necessary to allow the blood to clot in as thin a layer as possible, and then to stand the vessel so that the clot is vertical. The most convenient means of doing so is to use flat bottles (ordinary medicine vials) into which a number of thin pieces of drawn-out glass rod are placed. These thin glass rods hold the flat thin clot so that it will not slip down when the bottle is stood up. After 24 to 48 hours the serum is pipetted off and stored in sealed glass tubes or otherwise as desired.

By this technique more, sterile, clear serum is obtained from a given amount of blood than by any other means with which I am acquainted; and I would recommend its adoption on a larger scale by those who are interested in the preparation of serum. For the preservation of the

⁹ It may be added that as much as a cubic centimetre or so can often be obtained from the marginal vein of the guinea-pig's ear. This vein bleeds better than the larger veins of the ear.

serum I have never found occasion to add antiseptics, but it would appear that there is no reason against doing so, should it appear to be advisable, since for testing purposes it is rarely, if ever, necessary to use less than thousandfold dilutions, and also since, so far as clumping is concerned, the death of the bacteria to be tested is not a factor which introduces fallacies.

From numerous observations upon the course of the development of agglutinins in the blood, which I hope to publish in detail when I again have access to the records of my experiments, I am disposed to think that the effect of a given injection reaches its height (when killed bacilli are given) about 10 days or a fortnight after an injection. In following out this matter I have taken both slightly and rather highly immunised animals and removed samples of blood-serum at various periods after giving a further immunising injection. In all cases at least two samples have been taken previously to the injection so as to have ample control; samples were then taken either hourly or daily or at rather less frequent intervals and all were subjected to test with the same emulsion of bacteria. In order to gain some idea of the dilutions required for the estimation, four samples or so were tested at a number of dilutions in order to know what dilutions will be required for the final test. The tests are made by means of sedimentation in plain bulbous capillary tubes after the method devised by my friend Prof. Wright¹⁰ of Netley. With the large number often required it is impossible to use the microscope, whilst in an afternoon several hundred sedimenting preparations can be put up. The capillary sedimentation method has the further advantage of putting all the effects before the eye at once, so that the different tubes may be put side by side and compared with one another. Moreover, for testing the potency of a serum, the microscopic method has proved very unsatisfactory as well as laborious.

After using 10 pounds of glass tubing within a period of 3 months in the form of capillaries, I came to the conclusion that it would be advisable to adopt some means of cleansing them and putting them to further use. The following method has given satisfactory results and has not been guilty of giving rise to any accidental infection to my laboratory attendant: Two sufficiently deep wide jars, one filled with solution of potassium permanganate and the other with dilute (about 10 per cent) hydrochloric acid, are required. The attendant cuts off the lower (sealed¹¹) end of the capillaries with a file over the pot of permanganate,

¹⁰ *British Med. Journal*, 1897, i, p. 1214.

¹¹ It is not necessary to seal the upper end of the sedimentation capillaries.

into which each one is dropped vertically, whereby it becomes filled from below upwards with the disinfecting solution. After a day or so the tubes are removed, placed similarly in the acid and left there for a few days until the brown material is removed. They are then placed in a jar with syphon arrangement in which a constant slight flow of tap water gives them an automatic washing. When all the acid has been thoroughly removed they are shaken out and placed in a jar of distilled water (to remove lime salts) for a day or two, then they are again shaken out and allowed to dry. Lastly, they are put through the hot-air steriliser, after which they are ready for use.

For supports I keep a number of strips of sheet aluminum (about 1 inch broad and 18 inches long), each with a row of small holes (some 30-35 in number) sufficiently large to pass the capillary but not the fusiform bulbous part of the sedimentation tube. Each support is marked with a letter and each hole with a number, whereby the different specimens may be identified. When filled, the racks or supports are rested by their two ends upon a stand which formerly did service to support an incubator.

For making dilutions of small quantities and for making the mixtures of diluted serum and culture, the small porcelain pans, which are used for moist water colours, are extremely serviceable and, to my mind, more convenient than watch glasses.

Another small technical process which I have found most convenient may be mentioned. Given a sample of serum sealed in a glass tube, how is it most conveniently opened without contamination? Heat the end which it is desired to open to redness or nearly so in the flame and grasp it with the points of a pair of forceps which have been wetted with some antiseptic solution (*e. g.*, 2 per cent lysol); the end is immediately cut off and a sterile capillary tube, a number of which should be kept at hand in a sterile plugged test-tube, may be passed in and the required quantity of serum withdrawn. In this way I have removed samples of serum from the same tube over and over again without any accidental contamination; of course, after removal of sufficient serum, the point of the stock tube is again sealed.

II.—ON OTHER MEANS OF DIFFERENTIATING THE GROUPS OF BACILLI UNDER DISCUSSION.

From what has been already stated it is clear that the clumping reaction is of little value for differentiating and classifying these

bacilli in a satisfactory manner. It appears that we can only find whether the products of bacilli which are capable of giving rise to agglutinins are the same in two or more cases. Even then, although there may be the same substance or substances *qualitatively*, these may not necessarily be present *quantitatively* to the same extent. Again, by taking the same race of bacilli and its own serum, we find that the susceptibility of cultures made at different times is not necessarily the same. It seemed necessary, therefore, to enquire into other means of differentiation, but before discussing these, I propose to give a sort of classification of the types which have been studied, then to consider the ordinary characteristics which are used in laboratories, and, lastly, to give some account of media which may be useful for further work.

SUMMARY OF CLASSIFICATION.

Division I. Typhoid-like morphology: motile.

Order i. *Non-saccharid-fractors*, *i. e.*, do not ferment any saccharids.

Group A. Type, *B. faecalis alcaligenes* (Petruschky).

Order ii. *Dextroso-fractors*; *non-lactoso-fractors*: ferment dextrose and certain other saccharids, but not lactose.

Group B. *B. typhi abdominalis*: no evolution of free gas bubbles; CO₂ is formed by action on dextrose.

Group C. Type, *Bacillus "Gwyn"*; the gas liberated under favourable conditions from dextrose not limited to CO₂.

Group D. Type, *B. enteritidis*: gas liberated from dextrose not limited to CO₂, even in comparatively unfavourable conditions.

Division II. Colon-like morphology: motile.

Order i. *Dextroso-non-lactoso-fractors*.

Groups E, F, and G (not to be confused with *B. coli communis*): differentiable by nature of growth in lactose media especially.

Order ii. *Dextroso-lactoso-non-sucroso-fractors*. Group H. *B. coli communis* verus: do not ferment sucrose, but cannot be subdivided by present tests except serum-reactions.

Order iii. *Dextroso-lactoso-sucroso-fractors*. Group J. *B. coli communior*: differ from Group H in being able to ferment sucrose.

Division III. *B. lactis-aërogenes*-like morphology: non-motile. *Polysaccharid-fractors*. Includes *B. lactis aërogenes*, *B. pneumoniae* Friedländer, etc. Subdivision into groups requires further work. Ferment polysaccharids, such as starch.

DIVISION I. TYPHOID-LIKE MORPHOLOGY.

Group A. Type: *B. faecalis alcaligenes* (Petruschky). Culture kindly sent to me by Prof. Lehmann, who obtained it from Dr. Dieudonné. Under my observation about one year.

Rate of growth typhoid-like; does not ferment nor form acid in any sugary media; not agglutinated by highly potent (*i. e.*, thousand-fold dilutions) typhoid, enteritidis or coli serums, which are otherwise efficient dilutions.¹² Morphologically typhoid-like.¹³

Group B. *B. typhi abdominalis*.^{*14} Types: A number of different races have been utilized and some have been kept under observation for several years.

Forms abundant acid in dextrose media but no free bubbles of gas. Growth comparatively slow. Does not form any acid with lactose or sucrose. Gives clumping in high dilutions with potent typhoid serum. Sparse growth with characteristic acid formation in litmus milk whey.

¹² All the samples of human serum, typhoid or otherwise, which I have tested, clump this organism even up to hundredfold dilutions. It or some ally is probably a common inhabitant of the intestine of man, and its products are probably absorbed.

¹³ *B. fluorescens nonliquefaciens* has been taken for this organism, to my certain knowledge. *B. alcaligenes*, however, gives no trace of fluorescent pigment.

¹⁴ Serums were made from all types marked with asterisk *.

Group C. Types: *Bacillus* "Gwyn"* and *Bacillus* "O" of Dr. Cushing; kindly sent me by Prof. Flexner and Dr. Cushing.

Form abundant acid in dextrose media, but free gas only when the other constituents of the medium are favourable (p. 384). No acid or gas from lactose or sucrose. Rate of growth typhoid-like. In milk whey typhoid-like. Morphologically typhoid-like. No reaction with clumping typhoid, enteritidis, colon, etc., sera.

*Group D. Bacillus enteritidis group.*¹⁵ Types: Cultures of "Gärtner"* (see foot-note 14), "Günther,"* "Hatton"* (own), "Morseele," "Gand," "Sirault," "Calmpthoult," "Aertrycke," hog cholera, *B. typhi* murium, Psittacosis, morbificans bovis (of Basenau),* "Sheffield," "A" (own). (Unfortunately I was not able to procure cultures of Sanarelli's *B. icteroides* to compare with the others.) None of the above have been under observation less than one year.

Form acid and gas from dextrose. No gas or acid from lactose or sucrose. Rapidity of growth greater than typhoid; growth on gelatine in general distinguishable. Milk whey, characteristic turbidity, preliminary typhoid-like acidity (2-3 per cent), alkaline about fourth day. Serum reactions not universal within the group; three main subgroups are recognisable (see p. 356): (α) "Gärtner," "Morseele;" (β) "Hatton" and other races mentioned except (γ) *B. morbificans*. These subgroups run more or less into one another, thus "Günther" is more or less intermediate between (α) and (β); but there is no advantage in complicating too much.

Slight reactions with sera from certain races of typhoid, especially in the case of subgroup α . No reaction with colon, etc., sera. Morphology typhoid-like.

¹⁵ Owing to the confusion which has been made amongst the bacteria of swine diseases, I think it well to avoid speaking of a "Hog-cholera" group.

DIVISION II.—COLON-LIKE MORPHOLOGY.

Group E. Dextrose, but not lactose, fermenter. Type: "Urethra." Given to me as a typical *B. coli* communis; obtained in a culture made from urethra; under observation 5 years.

Forms acid and gas from dextrose, none from lactose or sucrose. Milk whey never goes acid but becomes turbid. Morphology colon-like. Rate and appearance of growths colon-like. No reaction with any of the sera mentioned; it is capable of reaction to its "own" serum, but none sufficiently potent was prepared for trial on other bacilli on an extended scale.

Group F. Dextrose, but not lactose, fermenters. Type: "425"* (see foot-note 14). Obtained by me from fatal case of perforative peritonitis; under observation 4 years.

Growth colon-like. Morphology colon-like. Forms acid and gas from dextrose. Slow and slight formation of acid with lactose but no gas. Milk whey becomes turbid, and acid corresponds (2.5 per cent) to that of a typhoid or enteritidis culture in milk, this, however, gradually increases instead of diminishing; milk is generally loosely clotted from 10th day. No acid or gas with sucrose. No reaction with sera of other groups mentioned. Reacts well with its own serum; no other type found which reacts with its serum.

Group G. Dextrose, but not lactose, fermenters. Types: "+ A,"* "FE₃,"* and 14 others; many obtained from contaminated water near Maidstone.

Acid and gas from dextrose; acid, but no gas, in peptone-lactose solutions; no acid or gas with sucrose. Morphology and rate of growth colon-like. In milk whey, after 24-48 hours, only about 5-6 per cent acid, milk whey does not become very turbid. By serum reactions fairly well-marked group, but some races which resemble the types fail to give serum reaction; members do not react with other sera than those of the group.

Group H. B. coli communis verus. Types: "Escherich 1"* and "2,"* "CN₂,"* "W"* and others. I have taken this to be the standard for *Bacillus coli communis*, from the fact that the type species were kindly especially examined for me in Prof. Escherich's laboratory and kindly sent to me by Dr. Pfaundler. "CN₂" and "W" were types from Prof. Gruber; so far as cultural and morphological characters go I am unable to distinguish them from Escherich's type.

Acid and gas from dextrose and lactose; *none* from sucrose. Milk whey turbid, with much acid (10-15 per cent), never becomes alkaline. Mutual reactions of sera absent. No reaction with other sera tried except type "G" of the following group, which reacts with "W" mutually.

Under this heading I may mention that several observers have kindly sent me cultures. One from Prof. Flexner agrees with the types; but one "Tübingen," used as a type for class purposes in Prof. Baumgarten's laboratory and brought to me by Dr. Fawcett in 1895, is certainly no *B. coli communis*. In general appearance on culture media and in fermentation tests it agrees fairly with the type, but in morphology it tends to grow in threads and the flagella are quite unlike those of any other colon-like bacilli; they are quite short and very closely coiled. One peculiarity is the tendency to aggregate in flocculi in broth cultures. If this is the culture used by Baumgarten as the foundation of his statement that a high clumping power of normal rabbit's serum upon *B. coli* is common, it gives some explanation of his statement by insufficient controls. "Tübingen" serum, which is potent to 200,000, has no effect upon *B. coli* ver., nor do any of the serums tried affect "Tübingen" cultures when carefully controlled against the flocculi formation. The bacillus has been examined from time to time during 5 years, and retains its original peculiarities.

Group J. Acid and gas from dextrose, lactose and sucrose. Types: "G,"* "TK,"* "CaV,"* "CaVI"* and many others—*B. coli communior*.

Characters and morphology like those of group *Bacillus coli communis* verus except that sucrose is fermented and acid freely formed from it. Mutual serum reactions not frequently met with within the group. I am inclined to think that this is a commoner inhabitant of human faeces than members of the last group, but have not made any direct experiments. Should this prove to be the case, it might be distinguished from the Escherich type as *B. coli communior*.

Note on non-motile colon bacilli.—Besides the bacilli directly conforming to Classes II and J, there are many that I have met which differ in apparent absence of motility and also want of flagella; in general, the majority I have studied are able to ferment sucrose. It is difficult to evaluate the true worth of this, to my mind slight, morphological difference or, indeed, to be perfectly certain that there is this difference. It may be that the actual "*B. coli immobilis*" is not real, for the conditions necessary for the full manifestation of motility are by no means determined. It sometimes happens that after long searches in hanging drops one or two really motile individuals may be found, although the vast majority are devoid of any true locomotor power. In searching for motile power it is necessary that the medium should not contain substances capable of giving rise to directly injurious matters (such as fermentable saccharids or alcohols) by the induction of acidity. It is generally stated that colon bacilli are sluggish, and this is no doubt true for ordinary media; I once succeeded in preparing a medium (containing horse's serum and human ascitic fluid) in which the types CN₂ and G whizzed across the field of the microscope fully as rapidly as a cholera vibrio; unfortunately typhoid and enteritidis bacilli were not tried in it. The matter was not followed out, but it appears to be evident that there is a good field for research in this direction.

Then, again, there are possible fallacies in the direction of the demonstration of flagella. The flagella of colon-like bacilli are far more difficult to demonstrate than those of other allied organisms. It seems that they are more readily shed and broken off than in the case of typhoid, enteritidis, etc., bacilli, especially when staining methods necessitating much washing and many transferences from one fluid to others are employed. Notwithstanding this defect, van Ermengem's silver method is, on the whole, to be preferred. To illustrate the fracture and disappearance of flagella by this method, I once made a batch of cover-slip smears from an emulsion (race CN₂) and stained some by van Ermengem and others by a method resembling that of Pitfield, but in

which saturated stanneous chloride was used in place of alum. (This gives very intense staining with the single fluid, but is apt to give very much precipitate, although I have sometimes obtained quite clear specimens. Formula: sat. sol. SnCl_2 , 1 pt., 10-15 per cent tannin 1 pt., sat. alcoholic methyl violet few drops; warm; put in the coverslips and allow to cool; wash in distilled water. I may note here that in methods like this all precipitate and indeed all stain may be removed by treatment with warm tannin solution and the specimen restained.) In the van Ermengem specimen the flagella were short and many of them broken off, whilst in the tin-method specimen the flagella were mostly retained and fully twice the length of those in the van Ermengem one. It is erroneous to state that the flagella of *B. coli* are shorter than those of typhoid bacilli; when complete they are fully as long. By repeated observations of apparently non-motile cultures I have occasionally succeeded in finding isolated flagella-bearing individuals. I am inclined to think that the absence of conditions favourable for the development and demonstration of flagella may lead to a false conclusion as to motility and the presence of flagella.

DIVISION III.—*B. LACTIS-AËROGENES-LIKE MORPHOLOGY.*

Division of B. lactis aërogenes.—Fermenters of polysaccharids, such as starch. Type: *B. lactis aërogenes*, "Kosseck" obtained from Prof. Escherich's laboratory, "Brad 1,"* "Brad 2,"* "Aërogenes"* from typhoid stools and many others.

Besides possessing the power of fermenting dextrose, lactose and sucrose, these groups can ferment also substances like starch and inulin. Some have very strong reducing power, and also great power of surviving and overcoming considerable amounts of acid which they have produced. They are non-motile, without flagella, and plumper than *B. coli*. They do not form chains or threads and are not often seen even in pairs. *B. mucosus capsulatus*, Friedländer's pneumobacillus, and also the "Schweinepest" bacillus used by Voges and Proskauer, are closely allied to this division. All of those which I have tested give the pink-red reaction of Voges and Proskauer,¹⁶ and this possibly may be regarded as a group colour-reaction: it is *not* given by any of the members of the other divisions which have been classified above. The bacilli are grown in a peptone-sugar-salts solu-

¹⁶ *Zeitschr. f. Hygiene*, 1898, xxviii, p. 30.

tion for a day or two and then about 1 cc. of strong caustic potash is added; an eosin-like colour appears after a while near the surface and lasts several days; the pink colour may appear as early as an hour after the caustic is added; Voges and Proskauer only describe its appearance after a day or so. The red colour may be quite intense and slowly fades away generally after several days.

There is no mutual serum reaction between the members tried nor with the other sera (typhoid, etc.) which have been prepared.

This group ("lactis aërogenes" of Escherich) is divisible into subgroups by the aid of fermentation tests, thus the "Kosseck," which, as coming from Escherich, may be regarded as "*B. lactis aërogenes* verus," is able to produce acid and gas from peptone-starch but not from peptone-inulin media. Other types will ferment both starch and inulin; others again inulin only. The power of fermenting starch no doubt has led to the description of the frothy culture on potato.

On gelatine plates the colonies of this group are bulky, white and moist, without the tendency to spread which characterises the colon-like organisms; consequently they are circular in outline. In slope cultures there may be a tendency for the growth to slide down when the surface is vertical.

BACILLI NOT TO BE CONFUSED WITH THE FOREGOING GROUPS.

One not infrequently meets with organisms which simulate the thin, spreading, irregular colonies with brown translucency of the colon and of the somewhat colon-like groups in gelatine plates. These, so far as I have observed them, are distinguishable either by morphological characters, such as chain and thread formation, or in cultures. Some of them are incapable of producing acid or gas with the three sugars, whilst others have some power in this direction. Some observers have gone so far as to include bacilli which are capable of liquefying gelatine under the colon group. This seems to me to be a childish disregard for one of the prime criteria of *Bacillus coli communis*. I have met with organisms which have the power of slowly liquefying gelatine media and which at first on gelatine plates had some resemblance both to *B. coli communis* and to *B. lactis aërogenes*. Any cultures about which there is some doubt should be kept on sugar-free gelatine media for 8-10 weeks, by

which time liquefaction will probably set in if it occurs at all. In general, I find these types do not produce acid in milk whey nor do they produce acid from lactose in broth. One culture of such an organism was kindly sent me by Dr. Mervyn Gordon; in his description of it in his paper,¹⁷ he states that it is agglutinated by typhoid serum. I have tried cultures of it with typhoid sera of potency 1:20,000-1:50,000 without the slightest trace of positive reaction at 1:100 and I have also prepared a serum with it of potency 1:200,000, which has not the slightest action upon several typhoid or enteritidis races even at 1:100 dilution. It is to be surmised that the technique adopted by him was faulty, as indeed is suggested by his description of his experiment.

REVIEW OF SOME TESTS ORDINARILY APPLIED.

Morphology.—Speaking generally, morphological characters are not of much value for subdivision of these bacteria. Among the organisms with typhoid-like morphology, I find that some individuals, as *B. morbificans bovis*, are not described as forming threads in cultures; in my hands this bacillus readily forms threads in broth and in agar.

The characteristic staining of the enteritidis group when grown at room temperature, especially upon gelatine, I have not met with in any other group or division. There is perhaps a tendency for the appearance of stained middle and unstained ends with the Bacilli of Gwyn and of Cushing, though I have not obtained very characteristic preparations such as are obtained with the enteritidis group.

It may be noted that flagella are of no value as a classifying test; it is not possible to differentiate between groups A, B, C and D by their means. The numbers of flagella given by many observers are not reliable, and are usually too low. The number of flagella will also not differentiate between the different groups of the more colon-like organisms. They are, therefore, of no value for ultimate classification.

Cultural Tests. Normal Agar and Gelatine Media.—The former does not afford any particular aid to differential diagnosis, although differences, such as the free growth of *B. aërogenes*, should be noted. The latter may give some idea of probabilities between the main

¹⁷ *Journal of Pathology and Bacteriology*, 1897, iv, p. 446.

divisions. By continued transference upon gelatine for many months or several years, as in keeping the collection growing, I find that the tendency to spread and form thin layers is frequently lost by the typhoid and sometimes by the more colon-like races; in general, the enteritidis has not much tendency to spread, although each of the races I have had does so from time to time. It should be noted that the gelatine for a slope culture must always be melted and sloped within a few hours or minutes of the time of sowing; gelatine which has been sloped for days becomes dry on the surface and this tends to prevent the spread of all but the most hardy races. Gelatine does not avail for discrimination between many of the groups.

Broth and "Peptones."—The normal broth I use is made from stale meat (beef or ox-heart) which has been hung for 3 or 4 days, infused for about 24 hours, strained, and heated after addition of 1 per cent Witte's "peptone" and 0.5 per cent pure NaCl. This is the basis of the gelatine and agar media. The neutralisation is done by caustic soda until a decided pink tinge is obtained with *rosolic acid* either on a porcelain plate or by cautiously dropping the dilute almost colourless indicator on the surface of a sample. The end reaction is practically at litmus neutral point. Phenolphthalein appears to me to be a clumsy indicator and the growths of the bacilli which I am dealing with do not appear to thrive nearly so well in media in the preparation of which this indicator is used. I have received several cultures from the Johns Hopkins laboratory and these, as well as others which have been sent to me from elsewhere, have given markedly more luxuriant growths in my hands than the original cultures from which they were planted; whether this is due to the reaction or the general constitution of the media is more than I can say.

In broth cultures of the enteritidis group, I have always found general turbidity of the medium and that a pellicular growth formed on the surface. In such broth no pellicle is formed by typhoid, "Gwyn" or colon groups. No pellicle occurs when simple "peptone" (Witte) and salt solutions (with or without sugars) are planted with any of the groups.

By digesting meat with pancreatic ferment (the "zymine" of

Burroughs and Wellcome was used), the resulting broth being made faintly alkaline (as above) to rosolic acid, most luxuriant growths occur with all the groups; and (except *B. lactis aërogenes*, which was not tried) abundant pellicle formation occurred. In this medium I find that typhoid races grow more freely than colon races do in "normal" broth. On the other hand, broth (and gelatine media made from it) made by digesting meat with pig's stomach and acid (L. Martin) is a very unfavourable medium for all these organisms; in general (including *B. lactis aërogenes*) they grow in floccular masses and leave the broth almost clear.

When broths for sugar tests—either the normal or pancreatic varieties—were made, they were first inoculated with *B. lactis aërogenes* and then with *B. coli communis* until no trace of acid or of gas was generated.

Milk.—Milk is not a satisfactory differentiator. It fails to distinguish groups A, B, C, D and E from one another, although these are so widely different. It also fails with H and J, early clotting occurring in both cases. Even when litmus is added it does not become much better, since owing to its initial alkalinity it does not shew differences markedly between A, B and E. Owing to alkali formation (after a preliminary diminution in some cases) a certain amount of clearing may occur (due to saponification?). This is especially the case with the enteritidis group, where it may occur after about 2 or 3 weeks; it may also happen with the typhoid group after a few weeks, as I have not infrequently seen, but it has generally occurred in tubes which have been left untouched in the incubator. Since the alkali formation is dependent upon growth in contact with the air, slight jarrings by removal may interfere with the phenomenon (compare the effect of movement in the preparation of diphtheria toxins).

If dextrose (1 or 2 per cent) is added, the milk becomes highly acid (*e. g.*, 10-15 per cent to normal alkali) with typhoid and enteritidis, but is not clotted. The clotting caused by *B. coli* is commonly ascribed to the acidity, but in this case it must be due to some special acid. According to Blachstein,¹⁸ *B. typhi* produce a lævo-

¹⁸ *Arch. d. sciences biol.*, St. Petersburg, 1892, i, pp. 199 and 299.

rotatory lactic acid from dextrose whilst *B. coli* gives a dextrorotatory one.

Potato.—Potatoes are too uncertain in their constitution (especially their acidity) to be reliable as a test. Moreover, it is impossible to distinguish by cultures on potato such widely different groups as E, F, G, H and J. Potato is therefore useless for purposes of discrimination.

Test Media of Capaldi and Proskauer.—The two media ((1), 2 per cent Witte peptone, and 0.1 per cent mannite, (2) asparagin medium with salts and glucose or mannite) recommended by Capaldi and Proskauer,¹⁹ are also of slight differential value. I find the peptone medium remains acid with groups B and C (typhoid and Gwyn) for several days, whilst it fails to differentiate between D, E, F, G, H and J. The other medium does only what is claimed of it for the typhoid group. After 24 hours' incubation, according to the vigour of growth, it soon becomes acid. I do not think that it is to be accorded any very high position as a test.

Indol.—The presence or absence of indol in cultures of these groups of bacilli is not of value for differential methods. Thus group G forms but a trace of indol, much like the amounts formed by typhoid and enteritidis. The most abundant indol production I have met is with *Bacillus* "425" (group F). There does not appear to be any relation between the amounts formed by the sucrose-fermenters and the non-sucrose-fermenters, nor between the lactose- and the non-lactose-fermenters, if we compare type "G" with "CN₂," or "Urethra" with a true *B. coli communis* of Escherich, in which the quantities of indol are about the same.

Semi-gelatinised Media.—These I have not tried. Since the designers have merely claimed that their action is due to the diffusibility of more highly motile organisms, it is not to be expected that groups A, B, C and D would be differentiable by this means.

Litmus Milk-whey.

Up to this point I have acted the part of a destructive critic, but I cannot speak too highly of this medium. It is largely upon it as a

¹⁹ *Zeitschr. f. Hyg.*, 1896, xxiii, p. 472.

basis that the present grouping or classification of these organisms has been made. In fact, according to my experience, more can be learnt from the course and appearance of cultures in this medium (titrated for acid if necessary with one-tenth normal NaOH) than *with any other single medium*. It is easy to prepare, but the method originally described by Petruschky,²⁰ who devised it, is not to be recommended in that he advocates the use of mineral acid, whereby some change of the lactose into dextrose and galactose might occur.

The following method gives admirable and apparently very constant results: Fresh milk (free from antiseptic adulterations) is slightly warmed and clotted by means of essence of rennet. The whey is strained off and the clot hung up to drain in a piece of muslin. The whey, which is somewhat turbid and yellow, is then cautiously neutralised, neutral litmus solution being used as an indicator, with 4 per cent citric acid solution. When it gives a good neutral violet colour with the litmus, it is heated upon a water-bath at 100° C. for half an hour or so; thereby nearly the whole of the proteid is coagulated. It is then filtered clear and neutral litmus is added to a convenient colour for titrations or rougher observation. Lastly, it is measured off by a burette into quantities of 10 cc. per tube or else sterilised at 100° C. in bulk. If difficulty is found in obtaining it perfectly clear it may be sterilised in bulk and allowed to settle for a few days or it may be passed through a Berkefeld filter; this, however, is rarely necessary to the practised laboratory attendant. *It must never be heated above 100° C.*

The value of this medium is partly due to the almost complete absence of proteid, to the presence of lactose and to the presence of a small quantity, perhaps about 0.1 per cent, of a sugar of the nature of dextrose or galactose (vide infra). Theobald Smith²¹ has recently called attention to the presence of the latter constituent in milk, and I may say that I came to the conclusion some while back that there was some other saccharid in milk besides lactose; the exact determination must be left to the chemist. The contained salts (which by the

²⁰ *Centralbl. f. Bakter.*, 1889, vi, p. 657.

²¹ *Journal of the Boston Society of Medical Sciences*, 1898, ii, p. 236.

way do not interfere with the colour or titration accuracy of the fluid) may also be of value in determining the results.

Group A. *B. faecalis alcaligenes* clouds the whey and produces alkali without previous formation of acid, and is thereby at once distinguished from members of groups B, C and D.

Groups B and C are much alike; after 24 to 48 hours at 37° C. the medium is hardly perceptibly turbid but has become distinctly acid (= about 3 per cent normal soda); the reaction after this remains about the same.

Group D begins with an acidity of from 2.5-4 per cent, the medium being made quite turbid. About the 4th day, at 37° C., it commences to become alkaline and may soon reach about 16 per cent normal H_2SO_4 .

Group E, type "urethra," never forms acid in whey. It is distinguished by growth in glucose media from A.

Why this organism does not produce any acid is not clear; possibly the second sugar is not dextrose or possibly alkali is formed too rapidly for the manifestation of acid.

Group F produces an enteritidis-like culture at first, but the acidity continues and increases.

Group G produces 5-6 per cent of acid; the culture is only slightly turbid in 24-48 hours and never becomes alkaline.

Groups H and J give at least 10-15 per cent of acid in 24-48 hours; the fluid is turbid and never becomes alkaline.

Group *B. lactis aërogenes* makes acidity and turbidity; in vigorous growths the colour of the litmus is completely reduced except a thin film on the surface; upon shaking and aëration the red colour appears.

These short notes will give some idea of the value of the medium. No artificial medium that I have tried has given such good results.

FERMENTATION TESTS.

Methods.—The apparatus for determining the production of gas bubbles which I described some time ago²² has enabled me to carry

²² *British Med. Journal*, 1898, i, p. 1387.

out a very considerable number of fermentation tests. The apparatus consists merely of a small test-tube inverted within an ordinary one; the medium is poured in and the process of discontinuous sterilisation causes the inverted tube to become completely filled. It is preferable to use fairly recently sterilised media, for when kept for a few weeks air becomes dissolved and may appear as a bubble in the collecting tube if acid is produced by the bacillus under observation, giving a false impression of gas production. In all these tests it is advisable to do considerable batches at a time, both of known and unknown organisms, in order to be sure that the medium reacts towards the known in the way it should. It is also not a bad plan to do a double set and leave one undisturbed in the incubator whilst the other is examined day by day. The routine of examining after 24 and 48 hours, and then on the 7th, 14th and 21st days, seems to be ample.

When only CO_2 is formed by destruction of a sugar (as in the case of glucose with typhoid) no gas collects in the collecting tube as a rule, but when the evolution is caught in full vigour some may sometimes be seen in the inverted tube. When both H and CO_2 are given off, some CO_2 collects in the tube; this may be absorbed by caustic solution if desired, but I find that a sojourn of about a week in the incubator has a precisely similar result in that all the CO_2 has disappeared. I have not made any estimations of the relative quantities of H and CO_2 in the fermentation tubes, since these would be of no value whatever. To obtain ratios of value, the *total gas* formed must be collected and extracted by vacuum over mercury before estimation. The fad of recording the ratios CO_2 to H, obtained with ordinary open fermentation tubes, appears to me to be a sheer waste of time and trouble.

FACTORS TO BE CONSIDERED IN APPRECIATING FERMENTATION TESTS.

Several factors have to be considered in appreciating the results of growths in media containing sugars, fermentable alcohols, etc.

It is of prime importance to know that the sugar employed shall not have been changed in its constitution during the process of making the medium. It is of no less importance to ensure that other con-

stituents of the medium are unable to give rise to fermentations. To ensure these points:

(a) No sugar-containing test medium should be exposed to the action of free acids (especially mineral acids) or caustic alkali.

(b) No sugar-containing test medium should be exposed to temperatures above 100° C.; indeed, the lower the sterilising temperature used the better.

(c) No sugar-containing test medium should be heated at all, except it be in a *neutral* condition, some such neutral point as that of litmus being used.

(d) No sugar-containing test medium should become yellower or browner than the stock solution to which the sugar has been added for the preparation of the medium; the change of colour shews that some change has taken place. (The medium called dextrose-broth, or dextrose-gelatine, seen in most laboratories is manifestly browner or darker than the plain media. This only means that the medium is not truly a dextrose-medium; the addition of dextrose to a medium should produce no change of colour if it is to be used for test purposes.)

(e) The stock solution to which the test sugar is to be added must be tried with appropriate cultures to prove the absence of fermentable matter. It should be ascertained that no acid, as well as no gas, is produced. This is especially necessary where meat-broths are used. It may be necessary to plant broth several times with enteritidis, colon, or aërogenes cultures before it is finally made up with sugar.

(f) Eggs should not be used for clearing media destined for fermentation tests, the ovomucoid and other constituents being capable of being split up.

(g) When tests are made upon sugary or fermentable media, they should be efficiently controlled by means of cultures of known bacilli in the same batch of medium.

(h) It is well to see that tested cultures are really pure and also that any given test gives the same result on repetition as it did on first trial.

Especially in the trial of media of unknown constitution with bacilli

of known proclivities, it is necessary to pay heed to the following facts, which are also not without importance for general purposes:

1. *The result depends on the nature of the sugar, etc., employed.* By means of the groups of bacilli we are dealing with it is possible to determine that certain sugars may be present or that they are absent. But they will not shew differences between certain sugars. Thus dextrose, lævulose, mannose, arabinose (a pentose), galactose and maltose (a disaccharid) give much and apparently fairly equivalent quantities of acid with races which are able to decompose dextrose (*e. g.*, typhoid) or acid and gas with races (enteritidis and coli) which are able to ferment dextrose. This statement is founded on titrations of 1 per cent solutions containing 1 per cent Witte's peptone; very exact titrations with equimolecular solutions have not been tried.

Mannite and dextrin also react like dextrose, in that those bacilli which will make acid or gas with one of the former will do so with the other; less acid appears to be formed from the dextrin, which was carefully tested with Fehling's solution to see that it was sugar-free.

The groups under discussion fail in a general way to distinguish between these different substances; group A forms no acid from any of them. This being the case, it did not appear to me to be advisable to follow the suggestion kindly given me by Dr. Ruhemann to compare samples of these sugars of different configuration or rotatory power. It may be that the finer discrimination or greater differentiation of these or other bacilli may permit of less coarse work and greater discrimination.

With dextrose, lactose and sucrose, and starch, inulin and glycerin in conjunction with the use of milk whey, much may be done in classifying the bacilli which belong to these groups; the morphological characters, the rate of growth in normal media, the non-liquefaction of gelatine and appearances thereon and the decolorisation by Gram's method not being omitted.

2. *The result depends upon the amount of the sugar present and the power of overcoming initial acid formation.* Capaldi and Proskauer²³ have shewn that some bacillary cultures tend to remain acid

²³ *Zeitschr. f. Hyg.*, 1896, xxiii, p. 452.

when small quantities of glucose or mannite are present. Thus typhoid cultures in 1 or 2 per cent peptone with 0.1 per cent dextrose, and, I might add, with maltose, etc., tend to remain acid; eventually, however, this bacillus is capable of overcoming the acid and the culture then becomes neutral and eventually alkaline. I find, similarly, with 0.1 per cent lactose and a *Bacillus coli communis*, or 0.1 per cent sucrose and a *Bacillus coli communior*, after 24 hours' incubation there is no acidity, the acid formed being rapidly neutralised. On the other hand, 1 per cent of either sugar with the appropriate bacillus yields a permanently acid culture. It is evident, therefore, that the amount of the fermentable substance present must be duly considered.

3. *The result depends upon the favourableness of the other constituents of the medium to which the fermentable substance is added.* It is not merely necessary to have a given fermentable substance in a medium, the remaining constituents must be sufficiently favourable to the growth of the bacillus to be tested.

For most of the groups a plain solution of Witte's peptone 1 per cent and sugar 1 per cent is sufficiently rich in nutritive material for gas production, given a bacillus which has the power of fermenting the sugar. In the case of organisms which are not able to flourish well in such simple media no gas may collect, although the sugar is fermentable. I have repeatedly tested *Bacillus* "Gwyn" at different times (and also *Bacillus* "O" of Cushing less often) for gas formation in 1 per cent Witte's peptone and 1 per cent dextrose. It appears from these oft-repeated tests that, although the medium is acidified, no gas bubbles collect; in fact, so far as appearances go, the cultures might well be of typhoid bacilli. Very different, however, is the result if 1 per cent of dextrose is added to sugar-free "normal" or "pancreatized" broth; here in both cases, besides the abundant acid, much gas is formed.

The action of *B. enteritidis* (a hardy race) upon glycerin may be in the same category. On repeated observation I note that only acid and no gas is produced by growth in 1 per cent Witte's peptone and 1 per cent glycerin. On the other hand, all the groups from E on-

ward have the power of producing gas from this simple medium. I find, however, that Voges and Proskauer,²⁴ in their paper on the bacteria of hæmorrhagic septicæmia, say that the hog cholera bacillus ferments glycerin added to their peptone and salt solution. While I have not observed gas formation with this bacillus growing in simple peptone-salt solutions containing glycerin, it may be that the more nutritive meat broth may enable the bacillus to produce gas from glycerin. This is a point which I was about to determine when I left England on the present expedition.

Further experiments of a like nature were contemplated to determine the simplest media compatible with gas formation by more and less hardy groups. Some preliminary experiments were made with proteid-free media, such as asparagin solutions and sodium-urate solutions. The latter give very pretty results if saturated solutions are used with sugars, for where acid is engendered the uric acid comes down in beautiful acicular crystals.

4. *Is the sugar altered by growth of a bacillus which does not produce acid or gas?* I have made experiments, for instance, by growing *B. fæcalis alcaligenes* in glucose, and, after awhile, planting the medium with *B. typhi*; abundant acid is then produced. Or after growing *B. enteritidis* in lactose media, subsequent planting with a *B. coli* gives rise to acid and gas. These and other experiments shew that even if the sugar is altered, the resulting bodies are fermentable by those bacilli which are ordinarily capable of so doing.

Preliminary Search for other Sugars and Fermentable Substances.

It seemed possible that there might be many substances of carbohydrate nature which would serve for further differentiation of these and other bacillary groups. After consulting Tollens' "Handbuch der Kohlehydrate" and Landolt's "Das optische Drehungsvermögen," the most useful mode of attacking the question appeared to be an empirical haphazard one.

Various substances, such as fruits, seeds, etc., were collected and extracts of these were made in the cold state when starch, inulin, etc.,

²⁴ *Zeitschr. f. Hyg.*, 1898, xxviii, p. 20.

were present or suspected. The extract was then tested for its power of reducing Fehling's fluid both before and after hydrolysing with a trace of dilute sulphuric acid. Plain neutral litmus solutions²⁵ were made according to the indications of the amount of sugar present. These were put into my fermentation tubes and tried with beer yeast and two members of each of the above groups for acid and gas formation. Great care was taken that no extract was heated except in a litmus-neutral condition. Again, other media were first planted with yeast and thoroughly freed from any substances which this could destroy before testing with the bacilli.

Among the sources of sugars which have been tried the following may be mentioned: Truffles, yeast, potato juice (starch-free), juice of Jerusalem artichoke, milk of ripe cocoanut, acorns, madder root, mangold-wurzel, carrots, fruit of *Cratægus oxyacantha*, *C. pyracantha*, *C. punctata*, *Rosa*, medlar, *Solanum nigrum*, *S. dulcamara*, tomato, egg plant (brinjal), *Physalis*, *Cytisus laburnum* seeds, *Digitalis* leaves, privet berries, holly berries, mistletoe berries, ivy berries, fir cones, *Abrus* seeds, bananas and many other sources. In several instances the hydrolysed, as well as plain, extracts were tested. A few glucosides (*e. g.*, *Quillaia*) have also been tried.

It was intended to go more fully into those substances which appeared to promise results, and to purify the fermentable substance. This I have as yet been unable to do. After purification it was proposed to retest in media made of favourable constituents.

I shall not go fully into the results obtained up to the present. The work upon this line was commenced early last year (1899), but was much interrupted by other calls upon my time. But anyhow, this mode appears to open up an enormous field for research both by the bacteriologist and the chemist. A few points of interest may be noted. In the first place the plain extract from the fruits, seeds, etc., may not have sufficient pabulum for fermentations by the bacilli; thus a plain extract of hawthorn gave no gas with group C and one of medlar gave the same result with groups C and D, although acid

²⁵ Much credit is due to my laboratory attendant, Mr. W. Mitchell, for the preparation of these media; he managed to produce beautifully clear media from the most unpromising materials.

was formed. When peptone or broth (sugar-free) was added to the medium free gas evolution took place when the same bacilli were cultivated in it. In fact, all through the work with many of the extracts the difference between groups C (Gwyn and Cushing) and D (enteritidis) was exemplified time after time, the former giving cultures like those of typhoid. A few substances deserve particular mention:—

Yeast-broth.—The first lot tried was made from baker's yeast on Spronck's formula.²⁰ Much CO₂ was formed by all the group types: in only Cushing, Gwyn and *aërogenes*, however, did permanent (non-absorbable to NaOH) gas result. This appeared to promise well as a differential test between C and all the other groups except *aërogenes*. However, on repetition, I was unable to obtain exactly the same result, although the first test gave a most striking and apparently unequivocal result. This requires further work.

Cocoanut milk.—This was obtained from the ripe cocoanuts we get in England. According to Tollens, it should contain the disaccharid, cane-sugar or sucrose. On testing with Fehling it gives no reduction until it has been hydrolysed. If this non-reducing sugar were really sucrose, it should not be attacked by any of the groups but "J" and *B. lactis aërogenes*. As a matter of fact, however, even the typhoid bacillus produces much acid and all the other cultures much gas as well. It is clear, therefore, that the sugar is different from the ordinary sucrose; it reacts towards these bacilli like dextrose or more accurately the disaccharid maltose.

Jerusalem artichoke.—The filtered juice contains a similar sugar in that it gives no reduction of Fehling till inverted. With the cultures it acts like the cocoanut sugar. The same is true of tomato, mangold-wurzel, *Solanum dulcamara*, etc.

Glucosamin, prepared from decalcified crab-shells by sulphuric acid, reacts like dextrose with the bacilli. With group C the addition of something more than plain peptone is necessary for gas production. I need hardly add that this substance is not attacked by pure cultures of yeast.

²⁰ *Annales de l'Institut Pasteur*, 1898, xii, p. 702.

Potato juice, starch-free, is not without interest. It contains a substance capable of reducing Fehling, but it is attacked only by *B. lactis aërogenes*.

Acorns contain a body which is fermented by the sucrose-fermenters, but is not attacked by the other groups. This substance is destroyed by English beer yeast so that no fermentation occurs with the sucrose-fermenters after the action of the yeast.

Gums, such as arabic, tragacanth and agar-agar, are not fermented by any of these groups until hydrolysed.

It would be idle to pretend to do more at the present time than to call attention to the wide field for research which is thus opened. Much of the information upon sugars in books on chemistry, especially in regard to the fermentations by yeasts, seems most unsatisfactory. It does not appear at all clear (vide Tollens) that in general pure yeast cultures were used nor indeed what species of yeast was used.

If this contribution should stimulate further and more accurate work, both bacteriological and chemical, in this interesting field, it will indeed be more than this incomplete account of my preliminary work on the subject can claim to merit.

Pará, Brazil, August, 1900.

REPORT OF A LABORATORY EPIZOOTIC AMONG GUINEA-PIGS, ASSOCIATED WITH GASEOUS EM- PHYSEMA OF THE LIVER, SPLEEN AND KIDNEYS, DUE TO BACILLUS MUCOSUS CAPSULATUS.

By R. G. PERKINS.

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During the summer of 1899, an epizootic broke out among the stock guinea-pigs belonging to the laboratory, in the course of which twenty-five animals were affected. Careful watch was kept over the stock in order that such animals as showed symptoms of illness might be at once isolated; in this manner the course and duration of the infectious process in the various cases could be carefully watched and noted.

The symptoms were similar in all cases; the animals ceased to eat, their hair became much ruffled, and their condition grew rapidly worse, culminating in coma, which existed for some time before death. During the coma there were intervals of muscular twitching, which in one or two of the animals were sufficiently marked to be called convulsions. In the animals autopsied as soon as respiration and cardiac impulse had ceased, so far as external examination could determine, the heart was found to be still beating very slowly. The heart as a whole, the separate divisions, and even strips of the ventricular walls, responded readily to stimuli for fifteen minutes or more after removal from the body, though no precautions were taken to prevent drying.

The duration of illness in the fatal cases, from the first symptoms noted to the time of death, varied between 12 and 48 hours, but by far the greater proportion died in from 18 to 24 hours after the onset of the disease. Two of the infected animals recovered after an illness of seven or eight days. In these cases the condition of coma was not reached, but it was over a week before the animals began to eat, and between two and three weeks before they recovered their normal weight and health.

Careful autopsies were made in all the fatal cases from 15 minutes to 24 hours after death. The bodies were kept in the cold chamber until autopsied, at a temperature well below the freezing point.

Summary of autopsy protocols.—None of the bodies showed any swelling after death, nor could emphysema be detected by external examination. Further examination of the skin and subcutaneous tissues failed to demonstrate any emphysematous areas. The glands of the groin and axilla were slightly swollen.

Thorax.—The pleural cavities showed no adhesions and no excess of pleural fluid. The lungs were normal except in one instance, where there was a lax consolidation of the posterior part of the right lower lobe. The pericardium and heart were uniformly negative.

Abdomen.—In 15 cases, or 65% of the animals autopsied, there was a well-marked peritonitis, chiefly of the sero-purulent type, though a few flakes of fibrin were usually to be found in the dependent parts.

The liver in every case showed marked congestion and cloudy swelling. In 14 animals, or 60%, there was a general gaseous emphysema of this organ which in 4 instances was so extensive as to resemble closely the "Schaumleber" described in cases of invasion by *B. aërogenes capsulatus*. No areas of degeneration were noticed macroscopically.

In 6 of the animals there was associated with this hepatic emphysema a like condition of the spleen. This organ was markedly congested in all cases. In 8, or 39% of all, there was gaseous emphysema like that described in the liver. In 2 of these cases, the emphysema was so extensive that the splenic capsule was distended until the organ was cylindrical in contour, resembling a small sausage.

The kidneys and adrenals showed no macroscopic changes other than well-marked congestion and cloudy swelling.

The gastro-intestinal tract contained much gas, but no lesions could be demonstrated.

Bacteriological examination.—Coverslip preparations were made at each autopsy from the heart's blood, the liver and the spleen, and from the peritoneum in cases with inflammatory exudate. The coverslips were stained in various ways, both with the usual aniline dyes and with Welch's capsule stain. In all instances of peritonitis, large numbers of organisms were found in the exudate, and in most cases the blood and organs showed a small number of bacteria of the same type. The organism uniformly seen in the stained smears was a short bacillus, with rounded ends, $0.5\ \mu$ to $3\ \mu$ in length, and about $0.5\ \mu$ in diameter. These occurred most frequently in pairs. Examination by the hanging-drop method showed absence of motility. Capsules were readily demonstrated by Welch's method. The organism stained easily with the aniline dyes, and decolorized rapidly when treated according to Gram.

There was no pleomorphism observed in stained specimens from the organs and exudates.

Cultures were made at each autopsy from the heart's blood, the liver, the spleen, and from the peritoneum when an exudate was present. The usual precautions were taken to prevent contamination, the surface of the organs being seared with a hot knife before the insertion of the platinum loop. Glycerine-agar slants were used, and from these, when the organism had grown out, transplantations were made into glucose-agar, glucose-bouillon, gelatin, milk, and on potato. With two exceptions, each of these being from a peritoneal exudate, the original tubes showed pure cultures of the organism to be described. In the two mixed cultures there were a few colonies of *Staphylococcus aureus* in addition to the characteristic bacillus. The tubes inoculated from the peritoneum showed a profuse growth, while in those inoculated from the blood and organs the colonies were fewer and often separate. The superficial colonies were round, soft, grayish-white, smooth in outline, and somewhat raised above the surface of the medium. Under the low power they were seen to be darker in the centre than at the margin, and finely granular. The type of growth in various media, using a number of cultures from different animals for comparison, was carefully studied, and proved to be similar in all cases.

Glycerine agar.—24 hours at 37° C. Profuse, grayish-white, moist porcelain-like growth, with wavy edges, distinctly raised above the surface. The water of condensation contained a rather heavy, flocculent sediment, and was markedly viscid. Some tubes showed gas-bubbles in the growth itself, and in all there was gas-formation along the lines of the stab, sometimes in sufficient quantity to raise the whole slant half an inch or more from the bottom of the tube. There was no liquefaction or discoloration of the medium.

Glucose agar.—24 hours at 37° C. The growth was almost exactly like that on glycerine agar, though the gas-formation was more marked.

Gelatin.—5 days at 22° C. The surface growth closely resembled that on agar, but was less profuse. The growth along the stab was slight, and at the point of inoculation there was a raised, nail-head growth, not extending far from the centre. There was no liquefaction nor gas formation.

Litmus milk.—24 hours at 37° C. There was complete coagulation in every case, with acid reaction. The beginning of the acid reaction was noted in about 15 hours from the time of inoculation. During 20 days there was no peptonization.

Bouillon.—24 hours at 37° C. There was diffuse cloudiness, with a heavy grayish-white, flocculent sediment. Later, the bouillon became very viscid, hanging in strings from the loop.

Glucose bouillon.—24 hours at 37° C. Fermentation tubes, containing 1% glucose bouillon, invariably showed gas-formation. Unfortunately the reaction to lactose and saccharose was not tested, as the cultures were inadvertently destroyed during my vacation.

Potato.—24 hours at 37° C. There was a profuse, moist, viscid growth, grayish in color, containing numerous gas-bubbles.

Indol reaction.—Peptone bouillon kept for ten days at 37° C. and tested with 10% sulphuric acid and 1% sodium nitrite for the formation of indol gave a slight positive reaction in all cases.

Morphology and staining reactions in coverslips from artificial media.—Stained specimens showed a pleomorphic organism, most frequently in the form of a short bacillus, with rounded ends. Special stains for capsules gave negative results, except sometimes with coverslips from litmus milk. The organisms stained readily with the aniline dyes, and decolorized rapidly with Gram's stain. Examination by the hanging-drop method failed to show motility.

Exclusion of other organisms.—In the cases which showed marked gaseous emphysema of the organs, control experiments were made by cultures and by animal inoculations, to ascertain the presence or absence of *B. aërogenes capsulatus*.

Anaërobic cultures in glucose agar were made from the liver and spleen, and kept in an atmosphere of hydrogen in Novy's jars for 48 hours. Examination of the tubes showed growths similar in all respects to those noted above, except that they were somewhat less luxuriant. No organisms answering in any way to the morphology or staining reactions of *B. aërogenes capsulatus* were seen.

Bouillon suspensions from the emphysematous livers were made and inoculated into the ear veins of rabbits. The animals were killed by a blow on the back of the neck after a short interval, and kept at body temperature for 48 hours. No emphysema or swelling was noticed, and no gas bacilli were found in coverslips. Cultures from the blood and organs of the animals showed the organism described in detail above.

Animal experiments.—Inoculations of 1 cc. of 24-hour cultures in bouillon into the peritoneal cavities of guinea-pigs which had never been exposed to the infection caused death in from 12 to 186 hours, with symptoms similar to those described in animals dying from the original epizootic. At autopsy there was in every case a sero-purulent

peritonitis, and the organs showed marked congestion and cloudy swelling, but none of the experimental cases showed any gaseous emphysema in either subcutaneous tissue or organs.

Further experiments were made on the two animals which had survived the original infection, to find out if any degree of immunity had been conferred by the previous attack. When they had completely recovered from the first infection, 1 cc. of a 24-hour culture of the organism described was injected into the peritoneal cavity of each. The animals showed no signs of illness on the succeeding day, and the inoculation was repeated, in the same manner and quantity. This was done on the third day also, but the animals showed no sign of any discomfort. In order to make sure that the organism had not diminished in virulence, control inoculations were made at the same time, from the same tubes, into guinea-pigs not previously exposed to the infection. These animals died within 24 hours with the same clinical symptoms, post-mortem findings and cultural results as described.

This observation agrees with that of Howard,¹ who found that by starting with small, non-fatal doses both of living and of sterilized cultures and filtrates of bouillon cultures of a bacillus of the *B. mucosus capsulatus* group, obtained from a case of hæmorrhagic septicæmia in man, both guinea-pigs and rabbits could be accustomed to withstand doses fatal to untreated animals.

Microscopic examination of hardened sections.—Portions of the heart, lungs, liver, spleen and kidneys were removed at each autopsy, and hardened in Zenker's fluid and in alcohol. Sections were stained by the following methods: (1) Hæmatoxylin and eosin; (2) methylene blue and eosin; (3) Van Gieson's picric acid and fuchsin; (4) Weigert's fibrin stain; (5) Lugol's solution.

Sections of the heart showed marked congestion in all, and cloudy swelling in most cases. The lungs showed marked congestion.

The liver showed extensive changes. There was enormous congestion, both of the hepatic and portal systems. The intralobular capillaries were distended so that the liver cells were often separated into bands and small clumps. In a large number of the vessels no abnormal elements were present, but there was much granular detritus, which stained deeply with eosin. Some of the capillaries were irregularly dilated, and their lumina were empty, or at most contained a small amount of granular material. Many of the central veins of the lobules as well as many of the hepatic veins were much wider than normal. Some of them

¹ *Journal of Experimental Medicine*, 1899, iv, p. 164.

contained granular detritus, but most were empty. The liver cells about these distended veins and capillaries were elongated and flattened. This change often affected several rows of liver cells about these spaces. Some of these cavities were very large, and occupied nearly the whole field of a Zeiss DD lens. In some of these abnormally distended vessels, fibrinous thrombi were found, the thrombus often occupying a portion of the lumen, so as to leave an empty space, round or oval in outline. The vascular changes above described were clearly due to the gaseous emphysema found in the gross sections of the organ. Scattered through the sections capillaries were found, containing fibrinous thrombi. The liver cells were cloudy, and often contained large fat drops; the protoplasm was granular, but the nuclei as a rule stained normally.

Scattered through the sections from two cases were areas of varying size, in which there was more or less complete necrosis of the liver cells, which in many places was typical coagulative necrosis. In some places the cell-protoplasm was hyaline in appearance, while the nuclei stained well and showed but little change. In other places both cells and nuclei took a deep eosin stain, though the outlines of the nuclei could be still readily made out. The necrotic portions bore no special relation to the lobules, though perhaps more of them lay at the periphery than at the centres of the lobules. They varied in size from those including only one or two cells to those as large as or larger than a single lobule; these larger ones were often markedly irregular in shape. In some of these areas both veins and capillaries were filled completely with either hyaline or fibrinous thrombi, in some of the latter of which leucocytes were seen. The liver cells in the immediate vicinity of these degenerated areas, especially of the smaller ones, frequently showed dropsical degeneration. In and about these areas there were numerous spaces similar to those noted in the blood-vessels. Sections treated with Lugol's solution failed to show any amyloid reaction.

Sections of the spleen showed marked congestion. There was a diffuse emphysema, and the vessels were distended as in the liver. The cells of the splenic pulp showed no special changes, and no areas of necrosis were seen.

Sections of the kidney showed marked congestion, extensive cloudy swelling and granular degeneration of the epithelial cells of the convoluted tubules. In many places the cell-outlines could not be made out, but the nuclei throughout the sections stained uniformly sharply. In some sections there was diffuse emphysema, and the vessels were dis-

tended as in the liver and spleen. No areas of necrosis or of cell-infiltration could be seen in any section.

Sections from the liver, spleen and kidney, stained by Weigert's method for fibrin, showed small amounts of fibrin in some of the vessels, but nowhere else, and no organisms of any kind.

Sections were stained with eosin and methylene blue to determine the presence of organisms decolorizing with Gram. In the blood-vessels, especially the veins, and in and about the emphysematous areas, numerous bacilli of from $1\ \mu$ to $3\ \mu$ in length, and about $0.5\ \mu$ in width, were seen. They frequently occurred in pairs, and capsules could often be seen.

The fact that they stained with methylene blue but not by Gram's method, as well as their morphology, confirm the results in the cultures, excluding the possibility of the presence of *B. aërogenes capsulatus* or other bacteria in the emphysematous areas.

The observations recorded in this paper are of especial interest in view of Howard's² report in a recent number of this journal of an instance in a human being of general gaseous emphysema with gas-cysts in the brain, formed after death, and due to a member of the *B. mucosus capsulatus* group (variety "aërogenes" of Strong). He further found that these two bacilli and three other bacilli of the same group and variety as the latter, obtained at autopsy from various lesions, caused general gaseous emphysema in the cadavers of rabbits most abundantly with, but also without, intravenous injections of lactose or glucose solutions before killing the animals.

There are three facts of special importance connected with the observations herein reported:

1. The spontaneous occurrence of a very fatal epizootic among laboratory guinea-pigs due to *B. mucosus capsulatus*.
2. The development of immunity of a high grade from otherwise fatal doses of the organism causing the epizootic.
3. The development of gaseous emphysema of various organs in 56 per cent of the animals either just after or 24 hours after death, due to the same bacillus, the usual cause of such emphysema, namely *B. aërogenes capsulatus*, being carefully excluded.

² *Journal of Experimental Medicine*, 1900, v, p. 139.

It is to be regretted that by an accident during my absence the cultures of the bacillus were destroyed before I had an opportunity of determining its exact position in the "mucosus capsulatus" group, according to the amount of gas formed in glucose, lactose and saccharose bouillon. There seems to be no doubt, however, that it belongs to Strong's³ "aërogenes" variety of the *B. mucosus capsulatus* group, on account of its great production of gas in the animal body, and the rapid coagulation of milk. This view is further supported by Howard's already cited observation in this laboratory of gas-formation in both human and rabbit cadavers, caused by bacilli of this variety.

³ *Journal of the Boston Society of the Medical Sciences*, 1889, iii, p. 185.

ON THE RELATION OF CHRONIC INTERSTITIAL PANCREATITIS TO THE ISLANDS OF LANGERHANS AND TO DIABETES MELLITUS.

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PLATES XXVII AND XXVIII.

CONTENTS.

	PAGE
Introduction	397
Congenital syphilitic pancreatitis	400
Chronic pancreatitis of the developed organ	404
Chronic interlobular pancreatitis	406
Chronic pancreatitis following obstruction of the ducts	407
Chronic interacinar pancreatitis.....	414
Chronic pancreatitis associated with hæmochromatosis.....	417
Hyaline degeneration of the pancreas.....	419
Diabetes mellitus.....	422
Summary and conclusions.....	427
Description of plates.....	428

INTRODUCTION.

In the pancreas are found certain structures whose nature is still obscure, the architecture of the organ being much more complex than that of the salivary glands which it closely resembles. Within the acini, and apparently in their lumina, are the so-called centro-acinar cells whose origin and function are as yet unexplained. P. Langerhans,¹ in the first important contribution to our knowledge of the histology of the pancreas, described groups of cells situated between the acini and differing markedly from those of the ordinary glandular type. These groups, usually round, are composed of small, irregularly polygonal cells with a round nucleus and homogeneous refractive cell body. Numerous observers have since described these structures, which are usually designated "islands of Langerhans."

¹ Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Inaug.-Diss. Berlin, 1869.

In injected specimens Kühne and Lea² found glomeruli composed of wide tortuous anastomosing capillaries between which lie the cells which Langerhans described. The ducts of the gland are not continued into these bodies.

Various opinions are held concerning the nature of these cell groups. Some observers have thought that they are follicles of lymphatic tissue scattered through the organ. Many believe that they have the same origin as the secreting elements of the gland and, formed during embryological life, persist thereafter and probably subserve some special function. Lewaschew³ subjected the pancreas to prolonged stimulation by overfeeding or by the repeated administration of pilocarpin, and thought that he was able to transform small groups of acini into typical interacinar islets, thus increasing their number at the expense of the secreting tissue. His experiments have not been confirmed.

It is not surprising that little is known concerning the function of structures whose nature is so little understood. Several writers (Laguesse,⁴ Schäfer,⁵ Diamare⁶) have suggested that they furnish an internal secretion which influences carbohydrate metabolism. The only evidence in support of this suggestion is contained in the short preliminary notice of Ssoblew,⁷ which has appeared since the completion of the present study. He states that after feeding animals on carbohydrates the cells of the islands become more granular. After ligating the duct of Wirsung in dogs the islands of Langerhans, he finds, are not implicated in the sclerotic process which ensues. He thinks that this fact explains the absence of glycosuria after ligation of the duct. In human cases I had observed similar resistance of the islands to the inflammation consequent upon obstruction of the duct. In the pancreas of two diabetics, Ssoblew was unable to discover islands of Langerhans.

² Untersuchungen a. d. physiol. Institute d. Univ. Heidelberg, 1882, ii, p. 488.

³ *Arch. f. mikros. Anat.*, 1886, xxvi, p. 452.

⁴ *Compt. rend. Soc. de Biol.*, 1893, 9. s., v, p. 819.

⁵ *Lancet*, 1895, ii, p. 321.

⁶ *Internat. Monatschr. f. Anat. u. Phys.*, 1899, pp. 155, 177.

⁷ *Centralbl. f. allgem. Path. u. path. Anat.*, 1900, xi, p. 202.

From a histological study of these structures in man and in lower animals, in injected specimens, and in glands stimulated by the administration of pilocarpin, I have reached the following conclusions^{7a}: (1) The islands of Langerhans are composed of cells having the same origin as those of the glandular acini but forming structures which are independent of the secreting apparatus and in intimate relation with the vascular system. (2) In the splenic end of the cat's pancreas they have a definite position within the lobule, each of which contains one of these structures. (3) In the human pancreas they are more numerous in the splenic extremity or tail than elsewhere. Similar variation in their number is observed in cats and dogs. (4) Prolonged stimulation of the gland does not, as claimed by Lewaschew, transform groups of acini into islands of Langerhans.

Embedded as are these bodies in the substance of the organ, they cannot readily be subjected to experimental conditions which do not equally affect the secreting structures. It is particularly desirable, therefore, to observe what changes they undergo under pathological conditions and, if possible, to bring such alterations into correlation with concomitant pathological phenomena. From such a study, heretofore little pursued, we may hope for suggestions of the function or anatomical relationship of these obscure structures.

Acute rapidly destructive lesions of the pancreas, for example hæmorrhagic pancreatitis, affect the various elements of the gland almost simultaneously, and complete disintegration of greater or less extent results. When the organ is attacked by the less active irritants which produce chronic inflammation the different histological constituents are given greater opportunity to exhibit differences in their ability to withstand the destructive process. The islands of Langerhans do not always show alterations corresponding to those which occur in the tissues of the acini about them, often persisting, though the adjacent parenchyma is destroyed. Moreover, while in some varieties of chronic inflammation they are but little implicated in the sclerotic process, in others they may be markedly affected. It becomes of interest, therefore, to study the relation of these bodies to the various forms of chronic pancreatitis that are distinguishable.

^{7a} *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 205.

The histological details of chronic pancreatitis have been, however, little studied and slight attention has been given to the classification of the various types. For the purpose of the present study it is desirable to adopt a classification, although it is based upon the somewhat limited material available.

That form of chronic inflammation which occurs during fetal life and is associated with other manifestations of congenital syphilis presents histological features which distinguish it from the chronic pancreatitis of adult life. It is a disease of the developing organ and may appropriately be first considered.

CONGENITAL SYPHILITIC PANCREATITIS.

Birch-Hirschfeld⁸ first drew attention to the frequency with which the pancreas is affected by congenital syphilis, and described the lesion so accurately that nothing had been added to our knowledge of it until the appearance of the recent article of Schlesinger,⁹ who has made a systematic study of the condition.

Birch-Hirschfeld found the pancreas affected in 13 of 23 cases of congenital lues, but subsequent observers have found the lesion much less frequently and, indeed, Birch-Hirschfeld,¹⁰ studying a second group of cases, found changes in the organ only 29 times in 124 syphilitic new-born. Schlesinger in six instances found the enlarged firm organ the seat of a diffuse interstitial pancreatitis characterized by proliferation of interlobular and interacinar tissue penetrating at times between the cells of the acini. This inflammatory new growth is followed, he thinks, by atrophy of the parenchymatous elements, which, though they do not exhibit appearances of degeneration, atrophy and disappear. The growth of interstitial tissue, he finds, has its origin about the blood-vessels, and the arteries are the seat of a syphilitic periarteritis, the adventitia being infiltrated with lymphoid cells. As the lesion progresses the capillary network about the acini disappears. Schlesinger has observed that the islands of Langerhans are neither invaded by the new growth of interstitial

⁸ *Arch. d. Heilkunde*, 1875, xvi, p. 174.

⁹ *Virchow's Archiv*, 1898, cliv, p. 501.

¹⁰ Gerhardt's *Handbuch d. Kinderkrankheiten*, iv, Abth. 2, p. 753. Tübingen, 1880.

tissue nor implicated in the atrophy which affects the cells of the acini.

The histological details which I have observed in two instances of congenital syphilis of the pancreas will be recorded. The relation of the islands of Langerhans to the inflammatory process is of interest.

CASE I.—Infant lived three hours. Length of body 40 cm.

Anatomical diagnosis.—Congenital syphilis; interstitial pneumonia; interstitial pancreatitis; splenic tumor; chronic perisplenitis.

Microscopic examination of pancreas.—The interstitial tissue is greatly increased at the expense of the parenchyma. The lobules, composed of a few acini scattered irregularly in dense cellular stroma, form groups separated by looser cellular tissue in which are situated the small veins and arteries. The smallest ducts, beset with acini along their course, terminate in a group of acini which, though much less numerous than those ordinarily forming a lobule, are of normal size and are composed of cells showing no evidence of degeneration. The interstitial tissue, particularly that between the groups of lobules and hence about the smaller vessels, is very rich in cells, which often form foci of dense cellular infiltration. Cells of lymphoid and of epithelioid type are numerous, but in even greater number, particularly about the blood-vessels, are round, oval or polygonal cells with eccentrically situated nucleus. They have the characteristics of the plasma cells of Unna. Cells with eosinophilic granules are also abundant and are of two types: (a) Mononuclear cells whose protoplasm is closely packed with large conspicuous eosinophilic granulations; (b) small cells whose nucleus is usually bilobed or trilobed; eosinophilic granulations of smaller size scattered throughout the cell body are most abundant about the nucleus.

A conspicuous feature of the histological picture is the presence of compact round masses of cells embedded in the interstitial tissue, which is usually concentrically arranged immediately about them. By the character of the cells which, polygonal in shape, are stained bright pink with eosin and by their arrangement in columns between which are capillary vessels, these structures are identified as the islands of Langerhans. Though they are embedded in the stroma, which separates widely the neighboring acini, they are not invaded by the inflammatory change. At times it is demonstrable, most conveniently in serial sections, that these islands are in continuity with the ducts and acini of the gland (Plate XXVII, Fig. 1). At the periphery of the island one of the columns projects beyond the general circular outline and is continuous with

epithelial cells which, staining less brightly with eosin, are arranged about a lumen and are in turn continuous with adjacent acini. In many instances, however, an island traced through a series of sections is found completely isolated in the fibrous tissue.

CASE II.—Infant lived four hours. Length of body 50 cm.

Anatomical diagnosis.—Congenital syphilis; pemphigus neonatorum; interstitial pneumonia; interstitial hepatitis and pancreatitis; splenic tumor.

Microscopic examination of the pancreas.—The interstitial tissue is greatly increased and the parenchyma is in very great part replaced by it, acini and groups of acini being widely separated. The new tissue is very cellular, but the cells are for the most part of the epithelial type and accumulations of round cells are not found. Plasma cells and cells with eosinophilic granulations are but rarely seen. The acini form small groups which may be regarded as primary lobules, though the acini composing them are much less numerous than those of a normal lobule. Islands of Langerhans are conspicuous as compact round masses of epithelial cells and are scattered abundantly throughout the organ. The fibrous tissue is often concentrically arranged about them and at times they lie completely isolated. Not infrequently, however, as in Case I, they are in continuity with the neighboring acinar tissue; a double row of cells is found to be continuous on the one hand with a cell column of the island, on the other with a small duct.

The preceding cases apparently represent different stages of the syphilitic lesion. In Case I proliferating fixed tissue cells are very abundant, while cells, in part at least of vascular origin, namely, plasma cells and eosinophiles, are numerous and the condition may be interpreted as the active stage of a chronic inflammatory process. In Case II, though interstitial tissue is more abundant and the persistent parenchymatous elements are more scattered, cells of the lymphoid type are few in number, while plasma cells and eosinophiles are almost absent. The process here is more advanced and is no longer active.

A conspicuous feature in both cases is the presence of numerous islands of Langerhans surrounded by newly formed stroma, but unin-
vaded by it. In many instances the islands are found to be in continuity with the secreting structures of the gland (Plate XXVII, Fig. 1.) A cell column of the island is continuous with a small

duct-like structure, which is in turn continuous with glandular acini. The lumen of the duct does not penetrate into the island.

Birch-Hirschfeld, finding the pancreas of syphilitic fetuses rarely affected unless they had survived the full period of uterine development, came to the conclusion that the condition has its onset during the last months of fetal life. Schlesinger, however, cites the cases of Müller and Mraczek, in which, at the 5th month of development, advanced lesion of the organ occurred, and from his own experience concludes that the pancreas may be affected as early or as late as other organs.

The pancreas arises as an outgrowth from the intestinal canal, and the development of its parenchyma takes place in a mass of mesoblastic stroma which is replaced as the growth of the gland proceeds. At an early period of development, for example at the 5th month of fetal life, the acini form small groups widely separated by embryonic connective tissue. In my two cases of syphilitic pancreatitis the parenchyma presents the appearance observed about the 5th month of development, with the exception that the islands of Langerhans, which are inconspicuous in the undeveloped organ, are marked features in the syphilitic pancreas. In neither of the syphilitic cases was it possible to observe degenerative changes in the cells. The acini form irregular groups containing much fewer members than ordinarily compose a fetal lobule, or, as in the developing organ, form dilatations upon the sides of the small ducts. It is conceivable, therefore, that the disease, like many syphilitic lesions, is one of the interstitial tissue and the changes in the parenchyma result not so much from a destruction of the parenchyma as from an interference with its growth. The similarity between the syphilitic and the undeveloped organ may be thus explained. The development of the individual cell, however, is not retarded and the islands of Langerhans are the result of an early cell-differentiation. In many instances the islands remain in continuity with the tubular structures from which they had their origin. Often, however, the connecting strand of cells is no longer discoverable, and the condition resembles that ordinarily observed in the organ at the end of fetal development.

CHRONIC PANCREATITIS OF THE DEVELOPED ORGAN.

Several types of chronic pancreatitis affecting the fully developed organ have been described, and with the experimental demonstration of a relation between the pancreas and carbohydrate metabolism numerous attempts have been made to distinguish a variety of the lesion constantly associated with diabetes mellitus. A classification of these various forms of chronic inflammation based upon etiological data, though desirable, would be, with our present knowledge, as unsatisfactory as a similar classification of the varieties of hepatic cirrhosis. From an experimental study Carnot¹¹ reaches the conclusion that pancreatitis may result (a) from mechanical cause, for example, obstruction of the pancreatic ducts, from the action (b) of toxic material or (c) of microorganisms carried to the gland by the blood, or by the lymph or by way of the duct. Such a classification does not aid in the interpretation of lesions observed at autopsy, the etiological factors concerned being in the majority of instances obscure.

In many examples of chronic pancreatitis fibrous tissue between the lobules is increased; in others the interacinar tissue shows marked proliferation; occasionally individual cells are apparently separated by strands of fibrous tissue. Corresponding types of inflammation have been described as interlobular, periacinar, and monocellular.

It has been thought that the increase of interstitial tissue may have at times a constant relation to the blood-vessels or to the ducts, being in part at least a proliferation of the connective tissue about these structures. Lemoine and Lannois¹² have described perivascular interstitial pancreatitis. From a study of four cases of chronic inflammation associated with diabetes they have thought that the new growth of fibrous tissue has its origin in the walls of the blood-vessels. They find about the vessels masses of sclerotic tissue sending processes between the acini and even separating the individual cells (*sclérose unicellulaire*). G. Hoppe-Seyler¹³ has described chronic

¹¹ *Recherches expérimentales et cliniques sur les pancréatites*. Thèse, Paris, 1898.

¹² *Arch. de méd. expér.*, 1891, iii, p. 33.

¹³ *Deutsch. Arch. f. klin. Med.*, 1893, lii, p. 171.

interstitial changes which he thinks are the result of arterial sclerosis. The parenchyma, he believes, undergoes degeneration as a consequence of disturbed nutrition, but no anatomical relation exists between the vessels and the new-formed tissue. Chronic pancreatitis in a case described by Rosenthal¹⁴ was accompanied by what he regards as alterations of the lymph-vessels, "lymphangitis proliferans," indicative, he thinks, of a probable syphilitic origin.

In the instances of chronic inflammation of the pancreas which have been available for my study, no constant relation has been discoverable between the new-formed tissue and the veins, arteries, lymph-vessels, or ducts, and there is no evidence that the process had its origin about these structures. Even where chronic pancreatitis follows obstruction of the ducts, sclerotic tissue is not more abundant about the ducts than elsewhere.

Two types of interstitial inflammation are, however, distinguishable. On the one hand, though the sclerosis is never accurately confined to one locality, it may be conspicuous between the lobules, the intralobular or interacinar tissue being little, if at all, increased. On the other hand the interlobular tissue may be only slightly altered, while fibrous tissue which replaces the parenchyma separates individual acini. In the first case the lobulation of the gland, which is normally obscure, becomes more conspicuous and wide bands of sclerotic tissue separate groups of lobules. The lobules are invaded in greater or less degree by the newly formed stroma and often entire lobules are in process of disintegration and replacement, but the progress of the lesion has been apparently inward from the periphery of the lobule. With the second type of chronic inflammation the lobulation of the gland is not accentuated, and the new fibrous tissue, primarily within the lobule, has a diffuse character, a network of irregular fibrous strands which vary much in thickness containing the glandular acini in its meshes.

The two types of chronic interstitial inflammation—(a) interlobular and (b) interacinar—characterized by the primary localization of the lesion, present other histological peculiarities. Of present interest is

¹⁴ *Zeitsch. f. klin. Med.*, 1892, xxi, p. 401.

the different relation which they bear to the islands of Langerhans, and it is desirable to study separately the changes affecting these bodies in the two conditions. The cases which have been studied exhibit individual differences and in a few instances the histological details will be briefly described.

CHRONIC INTERLOBULAR PANCREATITIS.

The sclerosis of the gland which follows obstruction of the ducts belongs to the interlobular type. Its definite etiology, as well as certain histological features, serve to distinguish it from other varieties of chronic pancreatitis whose etiology is more obscure. I shall first consider chronic inflammation of the interlobular type not caused by duct-obstruction. Six examples of this form of inflammation have been studied. Chronic inflammation, the result of duct-obstruction, will be subsequently considered.

In two cases (III and IV) the increase of interstitial tissue is moderate in amount and is most marked between the lobules, defining them more clearly than usual. Accumulations of lymphoid cells, among which are plasma cells and eosinophiles, indicate that the inflammatory process is still active. The lumina of the acini are widely distended, the secreting cells are much flattened and often show marked alterations; the scant protoplasm may no longer present a basal zone which stains with nuclear dyes, *e. g.*, methylene blue or hæmatoxylin, and the nucleus is often much swollen and irregular in shape and stains faintly. The islands of Langerhans are not the seat of similar changes; the cells composing them are normal in appearance, and though the acini are often separated by strands of sclerotic tissue, the islands are rarely, if ever, penetrated by this tissue.

In a third case (V) acute inflammation is associated with beginning proliferation of the interstitial tissue. The gland acini are distended and contain bacilli, morphologically of the colon type, and inflammatory products. The islands of Langerhans are apparently normal.

These three cases serve to direct attention to the fact that though the secreting acini have undergone marked degenerative changes, the islands of Langerhans may be unaltered. Anatomical peculiarities may explain the greater resistance of the interacinar structures to the

inflammatory process: (1) The vascular supply of the islands is richer than that of the adjacent acini. (2) Since the ducts do not penetrate them they are less exposed to the action of irritants which reach the gland by way of the duct.

In a fourth case of interlobular pancreatitis (Case VI) new fibrous tissue poor in cells outlines more conspicuously than is usual the gland lobules. The new-formed tissue penetrates the lobules and forms an intralobular network whose meshes are narrowest next the interlobular bands. Here the acini are atrophic in appearance. The islands of Langerhans surrounded by acini are, when the lobules are well defined, usually situated near their centre. The islands are therefore surrounded by the least changed acini and are themselves unaltered. Their very unusual abundance may be explained by the change in the parenchyma between them. The tissue being diminished in volume as a result of partial replacement by fibrous tissue, they are brought closer together and are more numerous in a given area.

Two cases (VII and VIII) represent advanced perilobular sclerosis. Wide bands of fibrous tissue, often containing lymphoid cells in great number, separate groups of lobules and send processes of new tissue into them. In places the glandular tissue is fairly well preserved, while elsewhere entire lobules are found in process of disintegration, the dilated and atrophied acini being separated by strands of fibrous tissue. The islands of Langerhans, scattered more abundantly than usual in the parenchyma and unaffected by the process, are surrounded by a delicate connective-tissue outline and are penetrated by delicate capillaries. Occasionally one finds an island surrounded by a few scattered acini, and though the neighboring secreting tissue has been almost entirely replaced by interstitial tissue, the island remains intact.

Chronic Pancreatitis Following Obstruction of the Ducts.

It has long been known that occlusion of the pancreatic ducts causes chronic interstitial inflammation of the gland. In human cases the usual causes of such obstruction are calculi or new growths, especially carcinoma, and numerous instances of pancreatitis follow-

ing such lesions are recorded. A large number of experimenters have produced sclerosis in animals by ligating the ducts.

Arnozan and Vaillard¹⁵ have studied the progress of the lesion in rabbits. The ducts soon become dilated, their epithelium proliferates, and cells are desquamated into the lumen. At the end of twenty-four hours the protoplasm of the secreting cells becomes clearer and the nucleus stains more deeply with carmine. At the end of about four days the swollen nuclei may almost completely fill the cell. At the end of seven to nine days round cells are numerous and before the fourteenth day connective tissue in large part replaces the parenchyma. The authors think that sclerosis is caused by the ferment present in the retained secretion.

Carnot¹⁶ produced interstitial inflammation by injecting the ferment papain into the duct. He thinks that several factors are active in the production of the sclerosis which follows obstruction of the ducts. The retained secretion, he believes, has a toxic action upon the parenchymatous cells. Obstruction to the outflow of fluid favors extension of infection along the duct from the duodenum. The action of infection plays an important part when occlusion is caused by calculus formation, a process which he thinks is of bacterial origin. Carnot suggests, moreover, that alterations of the reflex nervous stimuli which reach the obstructed gland are etiological factors in producing atrophy of the parenchyma. Such stimuli are no longer capable of exciting normal functional activity and, he thinks, cease to exert their influence on the metabolism of the secreting cells. The atrophy of the parenchyma may be somewhat analagous to the muscular atrophy which follows section of motor nerves.

Since the cells forming the islands of Langerhans have no communication with the lumen of the ducts and, presumably, play no part in producing the pancreatic juice, the possible factors mentioned would, if active, affect primarily the acini and only secondarily, if at all, the interacinar islands.

In the cases which I have studied varying degrees of atrophy and sclerosis have followed partial or complete occlusion of the pancreatic

¹⁵ *Arch. d. phys. norm. et path.*, 1884, 3. s., iii., p. 287.

¹⁶ *Op. cit.*

ducts. In two cases (IX and X) carcinoma of the gland was associated with chronic pancreatitis due to obstruction of the duct. In the first case (IX) retention cysts are numerous and changes are slight except in the immediate neighborhood of the largest of the cysts where dense fibrous stroma has replaced the glandular tissue. Dilated acini composed of flat atrophied cells are the only remnants of secreting tissue, while here and there are groups of epithelial cells, not differing from the islands of Langerhans present in the neighboring relatively normal parenchyma; they have withstood the sclerotic process. In the second case (X) the new growth has invaded the body of the pancreas and that part of the gland which is distal to the invading growth is alone sclerotic. Here the occurrence of an active chronic inflammatory process is shown by the presence of numerous lymphoid cells, plasma cells and eosinophiles in the interstitial tissue. Cell changes similar to those described by Arnozan and Vaillard are demonstrable. The most marked increase of fibrous tissue is between the lobules, but acini showing marked atrophic changes are often widely separated by new tissue. The structure of the islands of Langerhans is, however, unaltered.

In the following cases advanced chronic interstitial pancreatitis has followed obstruction of the pancreatic ducts:

CASE XI.—*Summary of clinical history.*—Female; aged 60 years.

Illness began about one year before death with symptoms of obstruction of the common bile-duct. At operation performed by Dr. Halsted¹⁷ a carcinoma of the bile-papilla and diverticulum of Vater was found and removed. The biliary and pancreatic ducts were transplanted into the duodenum. Subsequently an anastomosis was made between the gall-bladder and duodenum.

Anatomical diagnosis.—Recurrent carcinoma of the duodenum; metastasis in liver; occlusion of pancreatic duct; chronic interstitial pancreatitis; biliary fistula.

Pancreas.—On the left lateral wall of the duodenum is a crater-like ulcer with raised edges abutting upon the head of the pancreas. The pancreatic duct is included in the carcinomatous tissue at the base of the ulcer. The duct is greatly dilated and the pancreas is small and sclerotic.

¹⁷ This case is described by Dr. Halsted in the *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 4.

Microscopic examination of pancreas.—The parenchyma of the head and body has been almost completely replaced by dense fibrous tissue which contains fat in considerable quantity. Small isolated masses of glandular tissue still persist and are subdivided by penetrating strands of fibrous tissue. The stroma is in great part very dense and poor in cells. Here and there, however, round cells of the lymphoid type form large accumulations and mingled with them plasma cells are very numerous. Cells with eosinophilic granulations are present in the periphery of such foci and in the dense fibrous bands as well. The small ducts are dilated. The glandular tissue is in part normal in appearance, the gland-cells being little affected by the sclerotic process. In other situations secreting tissue is undergoing disintegration and the connective tissue not infrequently marks out areas which correspond apparently to lobules, but contain only a few atrophied acini composed of flattened cells about a dilated lumen. Here the inflammatory process is active; the connective tissue separating the atrophic acini is very cellular and contains many lymphoid cells, plasma cells and eosinophiles.

Islands of Langerhans, more abundant in the tail and body than in the head, are present in the relatively normal glandular tissue and are unaltered (Plate XXVII, Fig. 2). One not infrequently sees an island situated in the centre of a lobule which is undergoing disintegration. Scattered atrophic acini are separated by interstitial tissue containing large numbers of proliferating or exuded cells, but the island is normal in appearance and is not invaded by the newly formed fibrous tissue which surrounds it and isolates the much changed acini.

About an unaltered island may be found only a few acini to indicate that it was formerly embedded in the parenchyma, while elsewhere in the dense fibrous bands are seen isolated structures whose cells do not differ in character or arrangement from those of the interacinar islets. Such islands though surrounded by sclerotic tissue are not invaded by it and their cells which are normal in appearance form columns separated by delicate capillary vessels.

These isolated islands, however, finally undergo degenerative changes. They are diminished in size and often distorted. The cells, particularly at the periphery, crowded together, become smaller, and their nucleus also smaller than usual is often irregular in shape and is stained very deeply. Further changes are followed with difficulty since the much altered groups of cells are hardly recognizable as islands. Small groups of epithelial cells separated by strands of connective tissue probably represent a late stage of atrophy and precede final disappearance and replacement by fibrous tissue.

CASE XII.—*Summary of clinical history.*—Male; aged 43 years. Diagnosis: Pulmonary tuberculosis. No symptoms of diabetes were noted.

Anatomical diagnosis.—Chronic pulmonary tuberculosis; gelatinous and caseous pneumonia; miliary tubercles; tuberculous pleurisy. Cirrhosis of the liver with fatty degeneration. Parenchymatous and fatty degeneration of the kidneys. Splenic tumor. Pancreatic calculi; interstitial pancreatitis; parapancreatic fat necrosis.

Pancreas.—The duct of Wirsung is much distended by numerous calculi. The small ducts are also dilated and filled with fine gritty material. The gland-tissue has in large part disappeared and is replaced by interstitial tissue containing much fat in which are small opaque white areas of necrosis. The concretions give the reactions of calcium carbonate.

Microscopic examination of pancreas.—The parenchyma has been in very great part replaced by dense sclerotic tissue in which are scattered foci of round cells. The ducts are widely dilated and contain clumps of calcareous material. In the head of the organ are small areas of glandular tissue subdivided by interlobular strands of fibrous tissue which occasionally send projections between the acini. Occasionally gland-lobules are found in process of disintegration, tubular atrophied acini with dilated lumen being separated by new-formed interstitial tissue. Scattered in the sclerotic tissue, most abundant in a section from the splenic end of the organ, are round and oval clumps of cells arranged in columns between which, though the tissue about is densely fibrous, one sees delicate capillaries, often distended with red blood-corpuscles. These bodies, which have the characteristic structure of islands of Langerhans, present no similarity to secreting tissue in process of destruction. Occasionally an island has the appearance of being compressed and distorted.

CASE XIII.—*Summary of clinical history.*—Male; aged 50 years. The patient gives a history of alcoholic excess. His illness began five months before its fatal termination with symptoms of pulmonary tuberculosis which gradually increased in intensity. On admission to the hospital two months after the onset of his illness the urine contained 5.2% of sugar. When given a diet very poor in carbohydrates (v. Noorden's standard diabetic diet) sugar disappeared from the urine and reappeared only when carbohydrates were added—90 grms. of white bread to the daily diet.

Anatomical diagnosis.—Pancreatic calculi; chronic interstitial pancreatitis. Chronic pulmonary tuberculosis with cavities. Chronic dif-

fuse nephritis; large white kidneys. Anthracosis of lungs, spleen, and kidneys.

Pancreas.—The organ, which is intimately united to the adjacent structures, is much diminished in size and is tough and fibrous in consistence. The atrophy is most marked in its central part, which forms a narrow isthmus connecting the head and tail and consists of the duct and a small amount of fibrous tissue about it. The duct, slightly enlarged, contains viscid white fluid and a number of small, granular, gritty, yellow calculi, the largest being the size of a split pea. On section the tissue has a grayish-yellow color and small masses of parenchyma project prominently between thick bands of connective tissue. Minute opaque points suggest fat necroses.

Microscopic examination of the pancreas.—Sclerosis is far advanced and is most marked in the tail of the organ where glandular acini are almost entirely absent. The glandular tissue which persists occurs as compact masses rarely more than 2 or 3 mm. in diameter embedded in dense stroma. At the periphery of this relatively normal parenchyma are found lobules or portions of lobules in process of disintegration and replacement by the interstitial tissue. Here acini composed of low cells about a dilated lumen are scattered in the fibrous stroma. The fibrous tissue is in general poor in cells, but in many places, particularly about the large ducts, lymphoid and plasma cells are abundant.

In the masses of glandular tissue islands of Langerhans are present, and though of small size, are normal in appearance. Very numerous, particularly in the dense stroma of the body and tail, are masses of polygonal cells occupying conspicuous, sharply outlined, round or oval spaces in the sclerotic tissue. They do not differ from the islands of Langerhans found elsewhere. They are often situated almost side by side, separated by only a small amount of stroma, so that at times ten or twelve are seen in the field of the low power. Examination readily shows that they do not represent groups of acini in process of destruction. Very frequently in the fibrous tissue about these islands lymphoid cells and plasma cells are very numerous and it may be assumed that the inflammatory process is still active.

The persistent islands are finally involved in the general sclerosis. An increase of fibrous tissue occurs along their capillaries which become coarse strands subdividing the body into small masses of atrophied cells. One finds broad bands of dense fibrous tissue containing no epithelial elements or only an occasional compressed group of cells similar to those forming the islands.

In the preceding cases (XI, XII and XIII) advanced chronic pancreatitis has followed obstruction of the ducts. The organ is densely sclerotic, glandular tissue having been replaced in very large part by fibrous stroma. Small masses of relatively well-preserved parenchyma, little if at all invaded, are embedded in fibrous tissue which contains almost no epithelial elements. Areas are seen where disintegration of the glandular substance is in active progress and here lymphoid cells are present in large numbers. A striking feature of the process is the abundance of the plasma cells of Unna, among which are cells with eosinophilic granulations.

The scattered acini show atrophic changes similar to those previously described. The islands of Langerhans which occur in this altered glandular tissue are unchanged and, even though the neighboring acini are widely separated by inflammatory new growth, are uninvaded (Plate XXVII, Fig. 2). The secreting tissue about them finally disappears and they remain completely isolated in the stroma, not infrequently the only vestiges of parenchymatous tissue in wide sclerotic bands. In a section from such an area, isolated islands may be very numerous, and since the sclerotic tissue occupies less space than the acini which it has replaced, they appear to be much more abundant than in the normal glandular parenchyma.

As it is improbable that the vessels supplying the islands with blood remain unchanged in the indurated stroma, it can hardly be doubted that the nutrition of the cells suffers. The tissue growing older apparently contracts and compresses them; their cells become smaller, the nuclei are small, irregular, and stain deeply. They finally disappear, being replaced by fibrous tissue, which may contain an occasional isolated group of much atrophied cells or may be completely devoid of such structures.

The islands of Langerhans resist the sclerotic process which follows the damming back of secretion upon the gland, and finally suffer only when the acini are almost entirely destroyed and replaced by dense scar-like tissue. This is what we might expect when we consider that since the lumen of the duct is not continued into the islands it is hardly conceivable that they are concerned in the production of the

pancreatic juice, so that they are not exposed to its injurious action when the outflow is obstructed. The changes which the isolated islands undergo are, it appears, due to compression by the contracting scar-like tissue in which they are embedded and to alterations of their blood-vessels. Doubtless the rich vascular system of the parenchyma is in large part obliterated when the acini are replaced by interstitial tissue and, consequently, the network of vessels within the island, which freely anastomose with the adjacent capillaries, is, as the process advances, less freely supplied with blood.

CHRONIC INTERACINAR PANCREATITIS.

The type of pancreatitis, which may be conveniently designated "interacinar," is characterized by the presence of new-formed tissue within the lobules. The lesion is diffuse but somewhat irregular in distribution; at one point there may be a general thickening of the connective-tissue network supporting the acini, while elsewhere occur compact bands or small masses of stroma. Though the interlobular tissue is not unaffected by the inflammatory change, its proliferation is not a constant feature of the histological picture. The lobulation of the gland is not accentuated as with the interlobular type, but, on the contrary, is obscured, since masses and strands of new tissue within the lobules make inconspicuous the interlobular boundaries. This type is much less common than the perilobular form, and I have been able to study it in only three cases. One of these was associated with the condition of general pigmentation to which von Recklinghausen¹⁸ gave the name hæmochromatosis, and, differing slightly from the other two, it will be considered separately.

CASE XIV.—*Summary of clinical history.*—The patient gives no history of alcoholic excess. The present illness began twenty months before death with polyuria. Much body weight has been lost. A year and a half before death the spleen was palpable and hæmatemesis occurred at intervals. At this time the urine contained 3.5 to 3.8% of sugar. The patient was readmitted to the hospital four days before

¹⁸ *Tageblatt der 62 Versammlung deutscher Naturforscher und Aerzte in Heidelberg, 1889, p. 324.*

his death with ascites and dilated superficial abdominal veins. The urine contained 2.5% of sugar.

Anatomical diagnosis.—Chronic interstitial pancreatitis. Cirrhosis of the liver. Thrombosis of the portal, splenic and mesenteric veins; hamorrhagic infarction of the intestine. Acute serofibrinous and purulent peritonitis. Acute splenic tumor.

Pancreas.—The organ is small and firm in consistence.

Microscopic examination of the pancreas.—The interstitial tissue is greatly increased and is richly infiltrated with fat. Almost every acinus is in greater or less degree surrounded by fibrous tissue, but the lobulation of the parenchyma is not more distinct than usual. Details of structure are somewhat obscured by partial post-mortem change, but the distribution of fibrous tissue is clearly demonstrable particularly in sections stained with phosphomolybdic-acid hæmatoxylin (Ribbert's method). The connective tissue forms a network in whose meshes lie the acini; the coarseness of the fibrous strands and the size of the meshes which they form vary much and correspond to the greater or less alteration of the contained acini. In many places the glandular tissue of a limited area is almost completely replaced, being represented only by widely separated atrophic acini. The new growth of tissue, which is often conspicuous about the ducts and blood-vessels, bears no constant relation to these structures.

Islands of Langerhans are very abundant and are sharply outlined in sections stained with phosphomolybdic-acid hæmatoxylin, since fibrous tissue, concentrically arranged, forms coarse capsules, separating them from adjacent acini. There is, moreover, a proliferation of the connective tissue within them. Along the capillaries somewhat irregular, spindle-shaped, or elongated nuclei are more numerous than usual, and there is an increased amount of fibrillated material which gives the staining reactions of white fibres. The cells of the islands are often very small and their nuclei, diminished in size, stain deeply. They are closely packed together to form wide irregular columns. Not infrequently the interacinar fibrous tissue is much more abundant in the immediate neighborhood of the islands than elsewhere and here forms a close network of coarse strands with small meshes containing atrophied acini.

CASE XV.—*Summary of clinical history.*—Male; aged 47 years. The patient has used alcohol in excess. His health has been good until six months before death. On several occasions he has vomited blood. For three months the appetite has been poor, but thirst has been excessive;

polyuria has been present. The body weight has been fairly well retained. The patient was in the hospital five days preceding his death, during which time the urine contained 0.6 to 2.46% of sugar; acetone was present. He was dull, drowsy and at times delirious.

Anatomical diagnosis.—Chronic interstitial pancreatitis. Cirrhosis of the liver. Chronic passive congestion of the spleen. Ascites. Parapancreatic fat necrosis. Arterial sclerosis. Gangrene of the leg.

Pancreas.—Weight, 108 grms. The organ is firm, particularly at its splenic end. Here the lobulation is obscured, the texture of the gland-tissue is compact, and on careful examination of the cut surface minute opaque points are seen. In the fat within and about the organ are small opaque yellowish-white areas.

Microscopic examination of the pancreas.—Throughout the organ there is an abundant diffuse new growth of interstitial tissue which bears no constant relation to the blood-vessels or ducts or to the interlobular tissue but is between the individual acini. This new tissue is poor in cells and those which it contains have elongated spindle-shaped nuclei. It consists in great part of white fibres loosely packed together. In the meshes of the irregular network which it forms lie acini or small groups of acini which are often atrophic in appearance. Acini of large size containing many centro-acinar cells are seen.

In the tail, islands of Langerhans are abundant and often of very large size, corresponding apparently to the opaque points seen macroscopically. They are surrounded by new fibrous tissue which often forms a thick capsule and separates them widely from adjacent acini. They are, moreover, invaded by the new tissue which often forms coarse ingrowths along their capillaries (Plate XXVIII, Fig. 3). Elongated and spindle-shaped nuclei are somewhat increased in number, but the perivascular thickening is in great part produced by fibrillated interstitial material which, like white fibres elsewhere, stains with phosphomolybdic-acid hæmatoxylin and with acid fuchsin. All the islands are surrounded by dense sclerotic tissue, but some are only slightly invaded by the process. Where there is marked thickening about the capillary vessels, the epithelial cells are diminished in size and are closely packed together; the nuclei are small and stain deeply.

While with the interlobular type of chronic interstitial inflammation the islands of Langerhans are unaffected by the sclerosis and show changes only when the lesion has reached a very advanced stage, in the cases just recorded a new growth of tissue within the lobules and

between the acini invades the interacinar cell-islands. They are almost constantly surrounded by fibrous tissue, which forms, as it were, a capsule separating them from adjacent acini, which are themselves abnormally separated from one another. About the capillaries there is a proliferation of interstitial tissue forming coarse strands between the columns of cells.

In certain instances of the interlobular type, proliferation of interstitial tissue occurs between the acini, but is confined to the periphery of the lobule (Case VI). The islands of Langerhans, situated in the midst of the secreting tissue, often near the centre of the more or less clearly defined lobule, are surrounded by the least changed acini. The condition present in the interacinar type of sclerosis is of different character. In the immediate neighborhood of the island may be found the greatest proliferation of fibrous stroma and the acini, separated from it and from one another by coarse strands of white fibrous tissue, are more atrophic than those at a greater distance. When the inflammatory process affects primarily the periphery of the lobule and progresses toward the centre, the islands are affected only when the lesion is very advanced. When the change occurs diffusely within the lobule all parts are equally affected and the islands suffer in common with the acini. Indeed, it often appears that the favorite seat of the lesion is the immediate neighborhood of the bodies.

Chronic Pancreatitis Associated with Hæmochromatosis.

Chronic pancreatitis associated with the condition which von Recklinghausen described as hæmochromatosis was found, in the only case available for examination, to belong to the interacinar type.

In this disease an iron-containing pigment derived from the hæmoglobin of the blood is deposited in various cells of the body. The seat of most abundant pigmentation is the glandular organs, notably the liver, and hepatic cirrhosis is constantly associated with the condition. As the process advances chronic interstitial inflammation of the other organs ensues. The increasing accumulation of pigment acts injuriously upon the cells and finally causes their disintegration. Fibrous tissue replaces the destroyed cells and contains the pigment set free by their death. Pigment accumulation followed

by cell-death and chronic inflammatory reaction is readily observable in the pancreas to be described.

CASE XVI.¹⁹—Male; aged 55 years.

Clinical diagnosis.—Typhoid fever. The skin showed a deep bronze-like pigmentation. Glycosuria was not present.

Anatomical diagnosis.—Typhoid fever; ulcers in the ileum; bronchopneumonia. Hæmochromatosis; pigmentation of the liver, pancreas, heart, stomach, intestine, peritoneum, lymphatic glands, skin and testicles. Cirrhosis of the liver. Chronic interstitial pancreatitis.

Pancreas.—Weight 170 grms. The organ is firm in consistence. The cut surface has a uniform deep chocolate-brown color. Septa of fibrous and adipose tissue penetrate the gland.

Microscopic examination of the pancreas.—Interstitial tissue is much increased; in many places it defines the lobules, but as a rule it is diffusely distributed, occurring as irregular masses and strands separating small groups of acini or individual acini. A conspicuous feature is the presence of brown-yellow pigment giving the microchemical reactions of iron both in the gland-cells and in the interstitial tissue. The cells of the acini contain the pigment in varying amount; here and there are seen acini whose cells are distended with pigment-granules, their protoplasm being almost entirely replaced. Such cells often show evidence of degeneration; at times the nucleus has an irregular outline and stains very palely, while in many instances no nucleus is demonstrable. The fibrous tissue replacing the disintegrated cells contains free granules of pigment which are larger and more globular than those within the cells.

Islands of Langerhans are fairly abundant throughout the organ, but are most numerous in sections from the tail. They are constantly surrounded by a small area of fibrous tissue containing pigment in considerable quantity. Embedded in stroma they no longer possess a regular round or oval outline, but are irregular in shape and are penetrated by thickened fibrous strands which follow the capillary vessels. The cells, forming compact columns, contain numerous pigment-granules which when least abundant are situated in the portion of the cell most distant from the capillaries and hence tend to occupy the mid-line of the cell columns. The cells of the islands usually contain much more pigment than those of the adjacent acini. In preparations hardened in Flem-

¹⁹ I have reported this case more fully in *The Journal of Experimental Medicine*, 1899, iv, p. 279.

ming's solution fat can be found in many of the secreting cells, but is constantly present in the cells of the interacinar islets.

The new-formed fibrous tissue is diffusely distributed and bears no constant relation to the lobules. The lesion affects primarily the parenchymatous cells and shows no special tendency to involve those in the periphery of the lobule. The stroma, irregularly thickened between the acini, is constantly increased in the immediate vicinity of the interacinar cell-groups.

The alterations of the islands of Langerhans associated with the deposition of an iron-containing pigment, hæmosiderin, in the parenchymatous cells are as follows: (1) Pigment is abundant in the cells and tends to accumulate in that part which is most distant from the capillaries. (2) The cells undergo fatty degeneration. (3) The island is embedded in a capsule-like mass of fibrous tissue containing pigment granules. (4) Strands of similar tissue penetrate the island, following its capillaries.

HYALINE DEGENERATION OF THE PANCREAS.

In the study of lesions of the pancreas the greatest interest centres in their relation to the disease diabetes mellitus. Before discussing the possible relationship of changes affecting the islands of Langerhans to this disease, I shall describe a very remarkable lesion of the organ occurring in a girl, who, for two years before death, had suffered from diabetes. For the tissues from the case I am indebted to Dr. Flexner, who has kindly placed them at my disposal.

The pancreas is the seat of a lesion which obliterates the vascular supply of a considerable proportion of the parenchyma. Of special interest is the fact that the process, though not confined to the islands of Langerhans, has so completely altered them that in no part of the gland are they recognizable. That intact islands are not discoverable is surprising when we find a considerable proportion of the parenchyma very slightly changed.

CASE XVII.—*Summary of clinical history.*—Female; aged 17 years. As a child the patient has never been healthy and when 17 months old her parents state that she suffered with an abscess of the abdominal wall

near the liver. The onset of symptoms of the fatal illness occurred two years before death with extreme thirst and polyuria; sugar was found in the urine and has been constantly present in large amount until death. Record of the quantity has not been preserved. Upon diabetic diet the sugar diminished in amount but did not disappear. Marked loss of body-weight was not noted. Death occurred with coma which appeared suddenly and lasted hardly more than twenty-four hours.

Autopsy.—The only lesion noted was that affecting the pancreas. The entire organ was preserved for microscopic study.

Microscopic examination of the pancreas.—The organ is in large part self-digested and stained specimens have a blurred appearance, cell protoplasm and nuclei staining with almost equal intensity. In the tail, however, several areas where the tissue is well preserved give a clear histological picture of the lesions which are present. The interstitial tissue is increased only in localized areas. Throughout the organ, readily distinguishable even in the most digested portions of the gland, are very conspicuous, sharply defined, round, or oval, hyaline areas embedded in the parenchyma. They vary considerably in size. Where the parenchyma stains deeply with hæmatoxylin these bodies stand out conspicuously as almost completely unstained areas formed by a congeries of tortuous hyaline columns between which are compressed lines of cells apparently of parenchymatous origin.

Much clearer pictures are obtained in sections from the tail of the gland where self-digestion is least advanced. Here in preparations stained with hæmatoxylin and eosin these structures form sharply defined areas (Plate XXVIII, Fig. 4), taking a bright eosin stain in marked contrast to the general ground of glandular tissue, which contains many nuclei staining deeply. Their structure is as follows: Coarse, tortuous, hyaline columns separate strands of tissue, containing nuclei and representing in part at least capillary endothelium, from compressed rows of epithelial cells, evidently atrophied parenchymatous cells. The hyaline material lies immediately outside the capillary wall, between capillary and parenchyma. Occasionally the lumen of the capillary is visible and may contain shadows of red corpuseles.

The hyaline material has at times an indistinctly striated appearance, the striation being parallel to the course of the capillaries. A zone near the capillary endothelium, but not in immediate contact with it, often contains a deposit of calcium salts and stains deeply with hæmatoxylin. The epithelial cells between the tortuous hyaline columns form compressed rows varying in width. The cell-bodies are diminished in size and at times are hardly recognizable. The cells are usually arranged in

columns giving no indication of acinar arrangement, but rarely within such an area or more frequently at its periphery is found a double row of cells about a well-marked lumen.

The hyaline material does not stain by Weigert's method for the staining of fibrin. Reactions for amyloid were not obtained with specimens hardened in alcohol. When sections are stained with phosphomolybdic-acid hæmatoxylin, the hyaline takes a peculiar bright-blue stain in marked contrast to the deep blue-black of the fibrous tissue.

In general the parenchyma in which the hyaline masses lie is not markedly changed. The cells are somewhat smaller than usual and in material hardened in Flemming's solution are found to contain numerous fat droplets. The interstitial tissue is not as a rule increased. In the tail the parenchyma, representing several groups of lobules, has been almost completely replaced by the hyaline structures described, between which is fibrous tissue containing only a few atrophied acini composed of low cubical cells about a distinct lumen. Islands of Langerhans of normal structure are not found. The blood-vessels outside the hyaline areas show no change.

Microscopic examination of other organs.—The liver is normal in appearance; there is no increase of interstitial tissue and the blood-vessels are normal. In a section of the kidney a small collection of lymphoid cells is present at one point. Otherwise no change is noted.

The very remarkable lesion just described has apparently obstructed the vascular supply of a very large proportion of the gland-parenchyma. New-formed hyaline material is deposited between the capillaries and the parenchyma-cells (Plate XXVIII, Fig. 4). This material has a homogeneous hyaline appearance and stains deeply with acid dyes. The tissue which was studied was hardened in 95 per cent alcohol and the absence of reactions for amyloid was not conclusive. That the lesion is not this form of degeneration is shown by the absence of similar change in other organs which, much more frequently than the pancreas, are the seat of amyloid degeneration. I have found in the literature no reference to a similar lesion of the gland.

In the tail of the pancreas areas of hyaline transformation are larger and more numerous than elsewhere, involving at least two-thirds of the sectional area. Though the remainder of the parenchyma is in a fair state of preservation, islands of Langerhans are

not found. This fact is especially remarkable when we remember that the interacinar islets are normally most abundant in this part of the organ. It is evident, therefore, that the lesion implicates these structures, but that it is not confined to them is shown by the extent and abundance of the affected areas. Often they correspond in size and shape to the islands, but they may be several times as large. The occurrence of epithelial cells arranged about a lumen, particularly at the periphery of the altered tissue, shows that acini as well as interacinar islets are affected. In the head and body of the gland, areas of hyaline transformation are less abundant and smaller, usually corresponding in size to islands of Langerhans. Unfortunately, self-digestion of these parts of the organ prevents the recognition of very early stages of the lesion and their relation to the various histological elements.

Of present importance is the fact that the islands of Langerhans are destroyed, or at least isolated from their vascular supply, while a considerable part of the secreting parenchyma is not markedly changed. The occurrence of diabetes mellitus under these conditions is of interest and will be now discussed.

DIABETES MELLITUS.

The great amount of experimental and clinical study which has been devoted to glycosuria and diabetes has brought forth few hypotheses which are not still disputed. That the pancreas has an important influence on carbohydrate metabolism is no longer denied, and it is generally accepted that complete destruction of the organ in animals causes diabetes. The following facts concern the present study:—

(1) Extirpation of the pancreas in animals is followed by symptoms which are characteristic of diabetes mellitus in man. Extirpation of a very large proportion of the organ, less than an eighth or a twelfth remaining (Minkowski), is followed by diabetes of greater or less severity. That man is not an exception to this general rule is shown by a limited number of cases where, following operative removal of a portion of the organ, diabetes has ensued.

(2) Diabetes mellitus is in a considerable proportion of cases accom-

panied by diseases of the pancreas, chronic interstitial inflammation or, less frequently, acute inflammation. The frequent association of two relatively uncommon conditions is evidence that they bear some definite relation to one another.

(3) There is no evidence that pancreatitis is caused by the diabetic condition, and when diabetes accompanies changes in the gland which follow obstruction of the duct by calculi or are associated with malignant growth, conditions certainly not consequent upon diabetes, there can be no reasonable doubt, in view of experimental results, that diabetes is secondary and caused by the lesion of the pancreas. The same conclusion may be reached when chronic interstitial inflammation of the pancreas of obscure etiology is associated with diabetes.

It is well known that diabetes is not always associated with a demonstrable lesion of the pancreas. In the production of glycosuria many factors doubtless take part, and in the present immature knowledge of carbohydrate metabolism it is impossible to maintain that diabetes is always caused by disease of the pancreas. Nevertheless, the experimental and clinical evidence is sufficient to justify the assumption that when diabetes is associated with a destructive lesion of the pancreas, the latter is the cause of the first-named condition.

Chronic pancreatitis is not always or, indeed, in the majority of instances, accompanied by diabetes. Since experimental investigations have shown that in order to produce glycosuria it is necessary to remove a large proportion of the pancreas, we need not expect the condition unless a great part of the parenchyma has been destroyed or functionally impaired.

Various observers have attempted to define a type of pancreatitis peculiar to diabetes. G. Hoppe-Seyler²⁰ and Fleiner²¹ have described cases of the disease in which chronic interstitial inflammation of the organ accompanied general arterial sclerosis. Both writers think that changes in the vessels are followed by nutritive disturbances which cause degeneration of the parenchyma and its replacement by fibrous tissue. The condition, Fleiner suggests, is analogous

²⁰ Op. cit.

²¹ *Berl. klin. Wochenschr.*, 1894, xxxi, pp. 5, 38.

to the contracted kidney which is at times associated with general arterial sclerosis, and to changes in the liver, heart and brain following arterial disease. Lemoine and Lannois,²² as already noted, have studied pancreatitis in four cases of diabetes and have thought that the new growth of interstitial tissue has its seat of origin in the perivascular tissue whence fibrous processes extend between the parenchymatous structures. An important feature of the inflammatory change described by them is the penetration of fibrous strands into the acini, separating the cells and producing what they designate unicellular sclerosis.

Hansemann²³ has attempted to define a variety of pancreatitis always associated with diabetes. The organ is diminished in size and is flattened from before back. Its interstitial tissue is in continuity with that of adjacent structures and consequently the removal of the organ is more difficult than usual. The microscope demonstrates an atrophy of the parenchymatous elements which are in part replaced by new fibrous tissue. He thinks that the lesion is similar to certain forms of granular atrophy of the kidneys.

Should there be, as Hansemann claims, a type of pancreatitis peculiar to diabetes, that is, a form of inflammation impairing the internal function of the gland, glycosuria would not ensue until the lesion had reached a certain grade of intensity, and in its earliest stage the lesion would not be accompanied by diabetes. On the other hand, when chronic interstitial pancreatitis, whatever the type may be, has destroyed a very large part of the parenchyma, one may expect diabetes; the specific type, should such exist, would be associated with the disease at an earlier stage.

For the purpose of the present study it is pertinent to inquire what histological changes are associated with the occurrence of diabetes. When a lesion of the pancreas is the cause of the disease, is the condition dependent upon changes in the acini or in the islands of Langerhans or in both? Total destruction of the acini is often accompanied by destruction or alteration of the interacinar structures, and rarely,

²² Op. cit.

²³ *Zeitsch. f. klin. Med.*, 1894, xxvi, p. 191.

if ever, are the islands the seat of marked lesion while the acini are unchanged.

The islands of Langerhans are composed of columns of cells having no communication with the ducts of the gland, but in intimate relation with a rich capillary network. An analogous condition is found in the thyroid gland and in the adrenal. The pancreas, as do these organs, exerts through the medium of the blood an important influence on metabolism, and it has been suggested by several observers that the islands of Langerhans may furnish an internal secretion to the blood. Whether the gland furnishes some substance which aids carbohydrate assimilation or destroys some noxious product hindering it, is immaterial to the present study. Where diabetes is the result of pancreatic disease, do the islands exhibit lesions?

I have examined microscopically the pancreas from eleven cases of diabetes, and in four instances such marked change was found that one could not doubt the relationship of the general disease to the lesion of the organ. The limited number of cases makes far reaching conclusions impossible. Nevertheless, several facts of considerable interest appear.

Where (Case XVII) the pancreas was found to be the seat of advanced hyaline degeneration, the islands of Langerhans were universally involved in the process so that structures recognizable as inter-acinar islets were not discoverable. It is probable that the lesion had its origin in these bodies, though with its advance it has passed their limits. On the other hand, a considerable proportion of the secreting tissue, though the seat of fatty degeneration, was in a fair state of preservation and there was no hyaline deposit about its blood-vessels. Where the histological picture was not obscured by self-digestion, which is itself evidence of functional activity, the gland-cells were relatively normal in appearance. In this case fatal diabetes followed a lesion which had in great part obliterated the islands of Langerhans, though a considerable proportion of the intervening parenchyma was relatively intact.

Of eleven instances of chronic inflammation, classified as interlobular pancreatitis, in only one case (XIII) was the lesion accom-

panied by diabetes mellitus. Here glycosuria was of a mild type and disappeared when the individual was placed upon a diet from which carbohydrates were as far as possible eliminated. With this type of inflammation the islands of Langerhans are implicated only when the lesion has reached an advanced stage so that the organ may be markedly sclerotic while the interacinar structures are still unaltered.

Advanced parenchymatous degeneration and interstitial inflammation were found in three cases where obstruction of the ducts had been caused by calculi or by neoplasm (XI, XII and XIII). Diabetes of a mild type was present in one of these cases (XIII), and inflammatory atrophy of the gland was of an extreme grade. A very large proportion of the parenchyma had been replaced by fibrous tissue; the islands which persist are often embedded in dense sclerotic tissue containing no other epithelial elements and often show marked alterations. Compressed by the scar-like tissue, their cells are atrophied and thickened strands of stroma penetrate along their capillaries.

It is well known that obstruction of the pancreatic duct in human cases is accompanied by diabetes only when the consequent atrophy has caused great destruction of the gland. In animals it is extremely difficult, if indeed possible, to produce glycosuria by ligation of the ducts. These facts are explicable, should we assume that the islands of Langerhans, which resist the sclerosis, are the elements of the gland influencing carbohydrate metabolism.

In two of three cases, classified as interacinar pancreatitis, diabetes accompanied the lesion. Microscopic examination of the gland demonstrated less change than was observable in several instances of the interlobular type unattended by diabetes (Cases VII, XI and XII). Microscopic examination revealed the presence of advanced diffuse sclerosis. In accord with what one might expect from the diffuse interacinar character of the newly formed stroma, the islands of Langerhans are not spared but are surrounded and invaded by new tissue.

In one case of interacinar sclerosis (XVI) diabetes was not observed. The inflammation here accompanied the disease, hæmo-

chromatosis. This condition has been shown by P. Marie,²⁴ Anschütz²⁵ and Opie²⁶ to cause chronic interstitial inflammation of the pancreas, which, having reached a certain grade of intensity, is in turn followed by diabetes, the diabetes with pigmentation, or *diabète bronzé* of French writers. The pancreas in the case recorded weighed 170 grms. and the chronic inflammatory changes were not very advanced. The individual affected with hæmochromatosis died of intercurrent typhoid fever, and it is probable that his pancreatitis had not reached a sufficient intensity to cause diabetes.

SUMMARY AND CONCLUSIONS.

(1) Congenital syphilitic pancreatitis retards the development of the glandular acini but does not affect the islands of Langerhans. Embedded in the stroma, but not invaded by it, the latter maintain their continuity with the small ducts and acini with which they have a common origin.

(2) Two types of chronic interstitial inflammation affecting the developed pancreas are distinguishable:

(a) *Interlobular Pancreatitis*.—In the interlobular variety the inflammatory process is localized chiefly at the periphery of the lobule and implicates the islands of Langerhans only when the sclerotic process has reached a very advanced grade. When pancreatitis has followed obstruction of the ducts, the islands long remain unaltered though embedded in dense scar-like tissue.

(b) *Interacinar Pancreatitis*.—In the interacinar type the process is diffuse, invading the lobules and separating individual acini. The inflammatory change invades the islands of Langerhans.

(3) A relationship has been observed between lesions of the islands of Langerhans and the occurrence of diabetes mellitus.

(a) In one of eleven cases of interlobular pancreatitis diabetes of mild intensity occurred. The sclerosis, which in this case followed obstruction of the ducts by calculi, was far advanced and affected the islands of Langerhans.

²⁴ *Semaine méd.*, 1895, xv, p. 229.

²⁵ *Deutsches Arch. f. klin. Med.*, 1899, lxii, p. 411.

²⁶ *Journal of Experimental Medicine*, 1899, iv, p. 279.

(b) In two of three cases of interacinar pancreatitis, diabetes was present. The third case was associated with a condition, hæmochromatosis, which at a later stage is associated with diabetes, the result of pancreatic lesion.

(c) In a fourth case of diabetes, hyaline deposit between the capillaries and the parenchymatous cells had so completely altered the islands of Langerhans that they were no longer recognizable.

DESCRIPTION OF PLATES XXVII AND XXVIII.

PLATE XXVII.

Fig. 1.—Congenital syphilitic pancreatitis (Case I). Showing a cell-column of an island of Langerhans in continuity with a small duct.

Fig. 2.—Chronic interstitial pancreatitis following duct-obstruction (Case XI). Showing islands unchanged though embedded in sclerotic tissue.

PLATE XXVIII.

Fig. 3.—Chronic interstitial pancreatitis of interacinar type (Case XV). Showing the invasion of an island by the inflammatory process.

Fig. 4.—Hyaline degeneration of the pancreas (Case XVII).

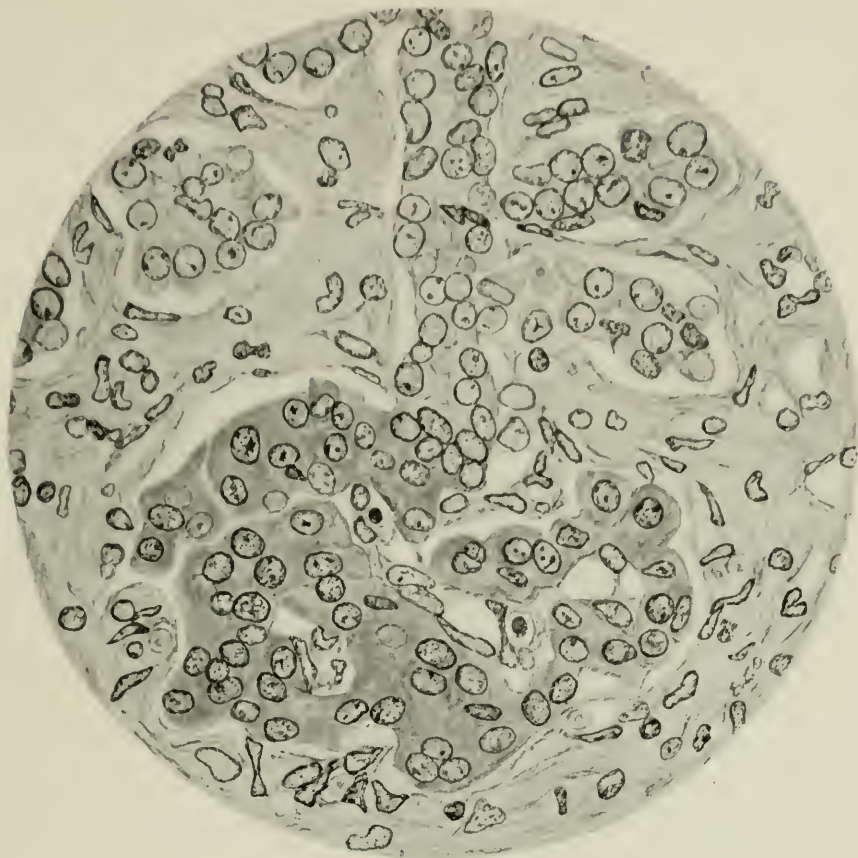


FIG. 1.

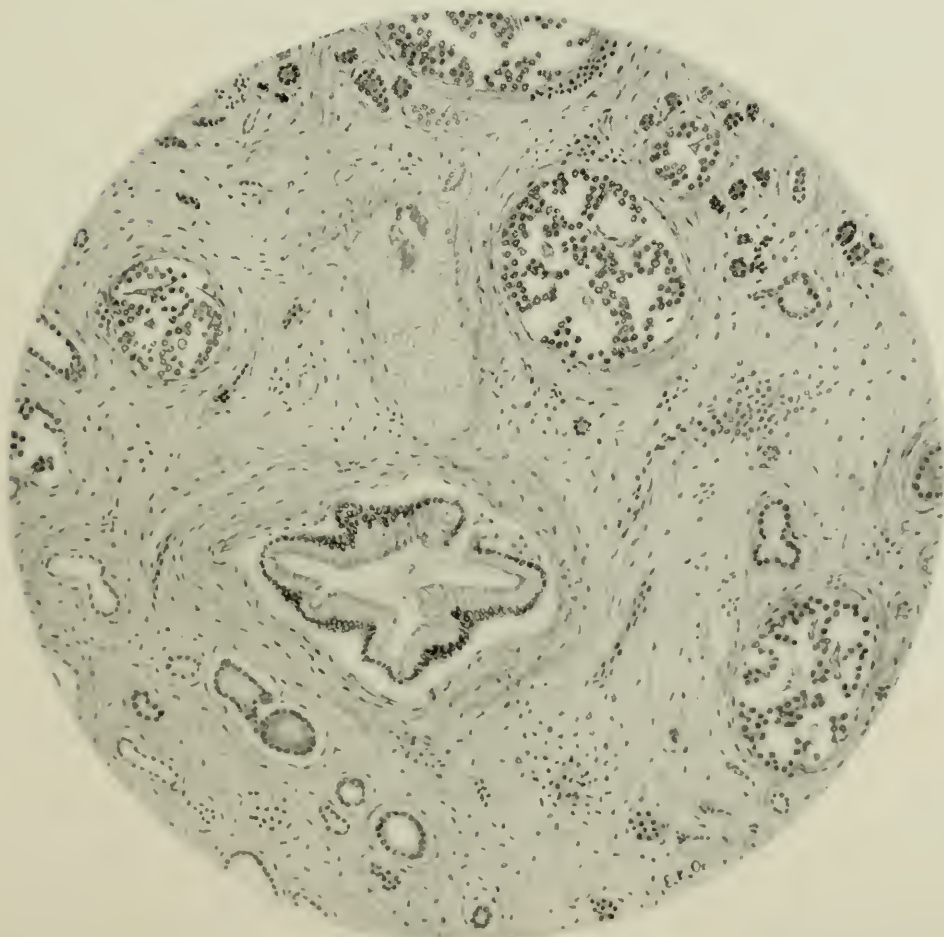


FIG. 2.

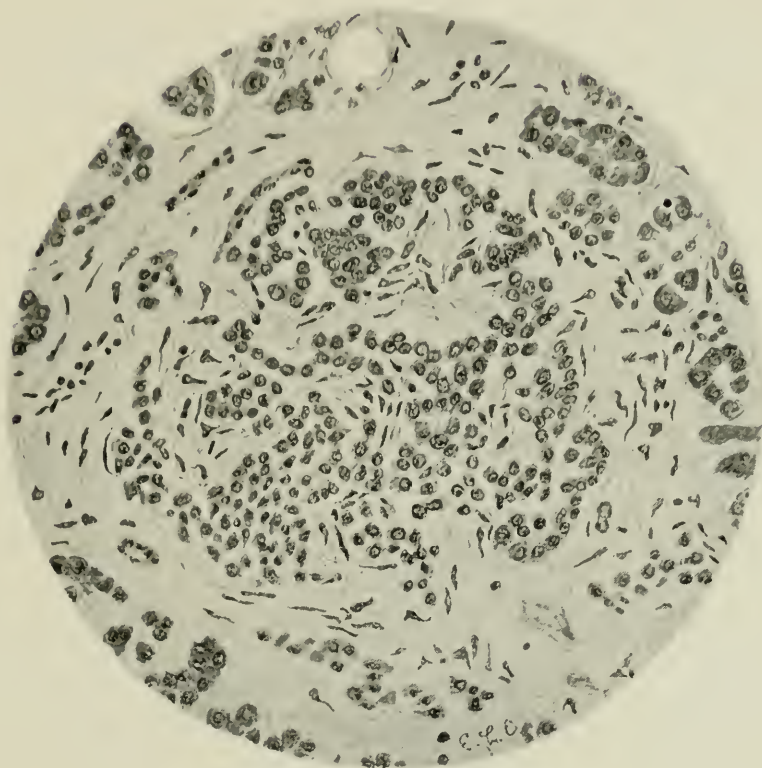


FIG. 3.



FIG. 4.

MALARIAL PARASITOLOGY.*

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PLATES XXIX-XXXII.

CONTENTS.

	PAGE
INTRODUCTION.....	429
I. TECHNICS.....	430
II. GENERAL MORPHOLOGY OF THE PARASITES.....	434
A. The tertian parasite.....	434
B. The quartan parasite.....	444
C. The æstivo-autumnal parasite.....	445
III. ON THE PLURALITY OF SPECIES IN THE ÆSTIVO-AUTUMNAL GROUP OF PARASITES.....	452
<i>Hæmamoeba immaculata</i>	459
IV. THE NUCLEAR BODY OF THE MALARIAL PARASITE.....	460
V. THE CRESCENTIC BODIES.....	466
VI. EXTRA-CELLULAR PARASITES.....	472
VII. ON A FORM OF CONJUGATION OF THE TERTIAN MALARIAL PARASITE.....	475
VIII. ON THE PLURALITY OF SPECIES OF MALARIAL PARASITES.....	482

INTRODUCTION.

Although the chief interest in the investigation of malaria centres at present in the study of experimental inoculations, of the natural mode of infection, and of the extracorporeal forms of the parasite, much yet remains to be learned about the morphology of the organism in its several varieties. The minute study of the morphology of the parasite has furnished far more cogent evidence of the existence of several species of the plasmodium of human malarial fever than has that of the clinical manifestations of the disease. Yet even the tantamount question of the unity or plurality of species is still far from satisfactory solution. Moreover, many details in the biology of the plasmodium of malaria, including the development of the crescentic bodies, whose significance has been partly determined, still remain

* Received for publication December 8, 1899.

obscure and require further observations on the occurrence and behavior of the parasite in the blood.

My observations on a considerable number of cases of Cuban malarial fever, seen at Montauk during the summer of 1898, although pursued primarily for the purpose of immediate diagnosis, bear on the topics mentioned. The conclusions reached at that time and more especially the deductions drawn from a more leisurely review of the material then obtained it is proposed to consider in the present article.

I. TECHNICS.

Experience with the technics of blood examinations in malaria has led me to restrict the use of fresh blood to the study of various vital phenomena in the parasite, such as amœboid movement, vibratory motion of pigment, and exflagellation. When parasites are scanty their discovery is so much more certain and rapid in stained dry specimens that a negative result with fresh blood invariably requires verification by search through a dry specimen, stained preferably by Nocht's method. Moreover, exclusive reliance upon fresh blood not only leads constantly to errors in diagnosis by beginners, but also has been the cause of many erroneous conceptions held in the past by experts.

The study of flagellate bodies may be conducted in fresh specimens prepared in the ordinary way, but placed by preference on a warm stage. The addition of a little water may facilitate the escape of the parasite from the cell and the formation of flagella. The successful action of water has been obtained by several expedients. Marshall added about an equal quantity of water to a small drop of blood containing many crescents and saw the almost immediate change of crescents to spheroidal bodies, followed by flagellation. Manson¹ recommends that the blood under the cover-glass be kept moist by exposure to steam exhaled from a hot, moist sponge. After a few minutes the cover may be carefully removed, the specimen dried, and the flagellate body stained.

Bignami studied in detail the flagellate bodies in specimens rather thickly spread, and prevented from drying in a warm moist chamber, while exflagellation occurred, with subsequent drying and staining. I find that a moist chamber may be conveniently secured in a Petri dish with tightly fitting vaselined cover. Wet blotting paper placed in the dish furnishes the necessary moisture. Specimens spread on slides or

¹ *Lancet*, 1896, ii, p. 1715.

covers may be kept moist for 10 to 20 minutes in such dishes, and flagellation proceeds with moderate rapidity.

Staining Methods.

1. *Eosin and Methylene Blue.*—For all ordinary purposes staining by eosin and methylene blue may be generally recommended, and was largely employed in the present cases. The solutions required are: (a) a saturated alcoholic solution of alcoholic eosin diluted with an equal quantity of 95 per cent alcohol, and (b) a saturated watery solution of Ehrlich's rectified methylene blue at least one week old.

A light staining by eosin, such as is given by the diluted solution of eosin, is essential for the clear demonstration of the parasite by methylene blue, and in specimens containing only the small signet-ring forms, heavy staining by eosin may almost entirely prevent the subsequent action of methylene blue, and these minute parasites may thus be overlooked.

2. Methylene blue fails to stain the young ring forms, especially those of the tertian type, as clearly as is desirable, and more powerful basic staining fluids may well be employed for this purpose. Nocht's method may be recommended over any other, as it facilitates the identification of the small ring by means of a large densely stained nucleus, but when this method cannot be employed, one may resort to the method modified by Fitcher and Lazear from the suggestions of Benario and Marchoux, as follows: Fix the specimens five minutes in 95 per cent alcohol, 100 cc., to which has been added 1 cc. of formalin. Stain one to three minutes in the following mixture: sat. alc. sol. thionin, 20 cc., 20 per cent carbolie acid, 100 cc. The fixing solution must be used fresh, and the staining fluid must be at least one week old. The rings are then densely stained and the specimens do not fade.

3. The sharpest demonstration of minute ring-shaped parasites was obtained in the present cases by staining one hour in diluted Gage's hæmatoxylin, before treatment by eosin and methylene blue. Hæmatoxylin stains the nucleus of the ring and makes the body of the parasite blacker after methylene blue. Such specimens are specially suitable for photography.

4. *The Nocht-Romanowsky Method.*—In 1890 Romanowsky published a method of staining the malarial parasite, which apparently demonstrated certain nuclear structures very imperfectly shown by other methods. The original description of this method directed that the specimens, fixed in equal parts of alcohol and ether, or by heat at

110° C., be placed for two to three hours in a fresh mixture made by adding concentrated (1 per cent) solution of methylene blue, 1 part, to 1 per cent watery solution of aqueous eosin, 2 parts. The red corpuscles were stained light pink, and the body of the parasite blue, while the chromatin particles of the nucleus appeared deep red.

The sketches accompanying Romanowsky's article were absolutely convincing that the new method was a most valuable addition to existing technical procedures, but it was soon found that the results obtainable from this method were extremely uncertain. We now know that only those who happened to secure particular specimens of methylene blue obtained successful results with Romanowsky's stain, and that the later highly purified products of the anilin-dye factories seldom contained the effective agent which united with the chromatin granules. Romanowsky believed that by the mixture of methylene blue and eosin a third compound was formed which stained the chromatin. Attention was not fully drawn to this probable explanation of the difficulty until Ziemann published the results of his experience with various specimens of methylene blue. After numerous attempts to stain by Romanowsky's method, following the exact directions, he sums up his results in the few words: "glückte kein Präparat." After a long series of experiments he found that a successful result depended upon the variety of methylene blue, the age of the solution, the quantity of eosin used, and the length of exposure. He secured most uniform results from the use of 1 per cent aqueous solution of methylene blue (med. puriss., Höchst) or Koch's methylene blue (Grübler), or Ehrlich's rectified methylene blue (Grübler). He found no difference in the effects of various specimens of eosin, but used principally a 1 per cent aqueous solution of watery eosin (Höchst). The proportions of these solutions required varied from 1 to 7 parts of eosin added to 1 part of methylene blue, and the time of staining from 15 to 40 minutes. The proportions of the dyes and the time of staining had to be determined for each specimen of dye. The older the solution of methylene blue, the less eosin was required in the mixture.

Gautier, who was very successful with Romanowsky's method, had the best results by using specimens of methylene blue marked C and BGN, and of eosin marked A, of the Baden Anilin factory. He found considerable difference in the results obtained with different specimens of eosin. Many other observers had a variable experience with the method, but it was generally agreed that a successful result was very uncertain and depended on factors little understood. I had few successful results in

following Romanowsky's method and just as many failures with Ziemann's procedures.

One turns with relief, therefore, to find that a modification recently suggested by Nocht furnishes a method which is invariably successful, without much dependence on the quality of dyes used, or the length of staining. Nocht's modification consists in the addition of a few drops of neutralized Unna's polychrome methylene blue (Grübler) to the 1 per cent solution of ordinary methylene blue. The usual specimen of polychrome methylene blue is distinctly alkaline and to be rendered effectual for the present purpose Nocht found that it required neutralization, preferably by acetic acid. This may be done by adding drop by drop of dilute 2 to 3 per cent acetic acid till the commercial fluid polychrome blue no longer turns red litmus-paper blue above the zone coming into immediate contact with the dye. I have never failed to secure a good result by the following procedure:

(1) To 1 oz. of polychrome methylene blue (Grübler) add 5 drops of 3 per cent solution of acetic acid (U. S. P., 33 per cent).

(2) Make a saturated (1 per cent) watery solution of methylene blue, preferably Ehrlich's rect. (Grübler), or Koch's, dissolving the dye by gentle heat. This solution improves with age, and should be at least one week old.

(3) Make a 1 per cent solution in water of Grübler's aqueous eosin.

The mixture is prepared as follows:

To 10 cc. of water add 4 drops of the eosin solution, 6 drops of neutralized polychrome blue, and 2 drops of 1 per cent methylene blue, mixing well. The specimens, fixed in alcohol or by heat, are immersed for two hours, specimen side down, and will not overstain in 24 hours. The density of the blue stain may be varied to suit individual preferences. The above proportions need not be rigidly followed, but the polychrome solution should be accurately neutralized.

Nocht later reports that the two solutions of methylene blue may be replaced by a 1 per cent solution of Ehrlich's rect. methylene blue, alkalized by a few drops of $\frac{1}{2}$ per cent of sodium hydrate, and left a few days in a thermostat at 50° C. This is the ordinary laboratory method of improvising polychrome methylene blue. With this preparation the procedure is as follows: To 2 cc. of water add 2 to 3 drops of 1 per cent watery eosin, and drop by drop of the alkalized methylene blue till the original red color of the eosin has almost disappeared. In this fluid specimens stain in 5 to 10 minutes. I have had little success with this method.

The rationale of the Nocht-Romanowsky method is not yet fully un-

derstood, but it appears most probable that a staining agent which unites selectively with chromatin exists ready-formed in polychrome methylene blue, and may be developed in specimens of methylene blue in various ways, among which is slow digestion with an alkali and heat. Nocht refers to this agent as "red from methylene blue." It is not the commercial methylene red, but may be extracted from polychrome methylene blue, etc., by chloroform (Nocht), and it is a reasonable expectation that it can be put on the market in pure form.

Nocht's method furnishes so much information regarding the minute structure of the parasite and renders the identification of the parasite so complete and positive that it must be recommended above all others. Moreover, it has a large field of application in the study of nuclear structures in various other microorganisms.

Goldhorn (N. Y. Path. Soc., Feb. 13, 1901) has recently succeeded in digesting polychrome methylene blue with lithium carbonate, neutralizing with acetic acid, so as to develop in it a large proportion of the red-staining principle. His method is as follows: Fix the preparation, which must be fresh, by immersion in pure methyl alcohol for 15 seconds. Wash in water and stain 7 to 30 seconds in 0.1 per cent aqueous solution of eosin. Wash and stain in digested polychrome blue 30 to 60 seconds.

Besides rapidly staining the chromatin of the parasite, Goldhorn's fluid demonstrates most exquisitely the early appearance of extreme granular degeneration of the infected red cell, in which respect it is a distinct improvement over any stain yet devised. The fluid can be obtained from various dealers in New York City.

II. GENERAL MORPHOLOGY OF PARASITES.

The Tertian Parasite.

The *youngest form* of the tertian parasite seen in the red cell is identical in appearance with the spore of the parent rosette. It is a compact spheroidal, or slightly oval, or irregular body, about 2μ in diameter. It shows an outer rim of basiphilic protoplasm enclosing a single large nuclear body, which is achromatic to methylene blue but stains readily in hæmatoxylin or by Nocht's method, and which is usually enclosed or accompanied by a clear achromatic spot, termed by Gautier "the milky zone" (Plate XXIX, Fig. 1). In the fresh condition these bodies are noticeably refractive, especially the nucleus,

change their position but rarely their shape, and are never pigmented. From the earliest period of infection the red corpuscle is often swollen, and exhibits advanced granular degeneration.

Within a few hours after the chill the parasite is usually found to have assumed a somewhat characteristic ring shape, which it commonly maintains in some definite form up to the presegmenting stage (Plate XXIX, Figs. 2-11). These ring-shaped bodies measure from 3 to 4 μ in diameter, and the regular ring form is retained, without marked increase in bulk at any point, for 6 to 8 hours. Sometimes the ring is elongated, one arm reaching across the cell, while a thin bow persists. Occasionally the ring appears to unfold, and the parasite stretches clear across the swollen cell, with the nucleus at one end. The tertian ring is rarely as geometrical or delicate as is the æstivo-autumnal signet ring. The development of pigment was inconstant in the present cases, some large rings failing to show pigment, but usually one or more fine grains were to be seen in the medium-sized and smaller forms. The ring always encloses a considerable mass of hæmoglobin. The nuclear body of the tertian ring is its most characteristic feature, appearing as a rather large, achromatic, highly refractive body, after methylene blue, but staining intensely with hæmatoxylin and by Nocht's method.

Significance of the Ring Form.—In regard to the formation and significance of the ring opinions are at variance. Most of the Italian writers hold that the ring form is not really a ring, it being bridged across by a transparent and vesicular nucleus. There are many considerations favoring this view, especially the usual appearance of the parasite in the fresh condition, and the fact that the chromatin usually lies within the ring, eventually filling it. On the other hand, Mannaberg and Ziemann claim that this body is a true ring, formed by the thinning of the central portion of the body, and state that they have seen the ring develop in this manner in fresh blood. From the examination of the rings themselves I have been unable to convince myself as to which view is correct, but there are some early forms of the parasite which strongly indicate that the ring does not represent a vesicular nucleus. In one such form the ring is unfolded

and the nucleus lies naked in the hæmoglobin. Elongated forms of the young parasite are often seen in which the ring is absent and the nuclear body lies bare at one end. These forms vividly recall the appearance of *Amæba coli*, in which the nucleus remains at the hinder end during active movements of progression. Further, it is difficult to associate the relatively huge size of the ring with any nuclear structure, as this would require the young malarial parasite to have a nucleus which is larger than that of the adult *Amæba coli*. Again, secondary rings sometimes form from the union of pseudopodia, and these are identical in appearance with the primary ring but lack the chromatin granules (Plate XXIX, Figs. 6 and 8).

In specimens stained by Nocht's method the chromatin is usually found within the ring, sometimes lying in an isolated position in the centre, but very often the chromatin is found *outside* of the ring, connected by a very fine thread of protoplasm. If the ring represents a vesicular nucleus, we have here the anomaly of a complete separation of chromatin from the vesicular portion of the nucleus, which is opposed to some rigid histological principles. Even more frequently the chromatin is found to be enclosed by bluish staining protoplasm which shuts it off entirely from the ring (Plate XXIX, Figs. 7 and 8).

From various biological studies it appears that the nuclei of the protozoa are usually widely different from those of the metazoa; that many protozoa do not have a vesicular nucleus, with cell membrane, linin, nucleolus, etc., but possess the so-called "distributed nucleus" composed of a number of granules lying free in the body of the parasite. The study of the malarial parasite by Nocht's method indicates that the nucleus of this protozoon is of the *distributed type*, which does not exhibit a vesicular structure nor possess a nuclear membrane.² On these grounds I am inclined to agree with Mannaberg and others who hold that the form in question is a true ring—a form usually, but not necessarily, assumed by the parasite—and does not represent a vesicular nucleus.

Comparison of the Tertian and Æstivo-autumnal Rings.—From the study of the ring-shaped tertian parasite and the æstivo-autumnal

² See Calkins on "Protozoan Nuclei" in *Annals N. Y. Acad. Sciences*, 1898, xi, Part iii.

signet-ring forms, in typical cases of these infections, it appears to me that these parasites, with very rare exceptions, can be fully distinguished from each other, even in this early stage, by the following peculiarities:

(1) The nuclear body and chromatin mass of the young tertian parasite is achromatic to methylene blue, which densely stains the nucleus of the *æstivo-autumnal* organism. I have been unable to find in the literature any specific reference to this peculiarity as a diagnostic point, but it may be readily verified by comparing specimens of the two parasites stained by methylene blue and by Nocht's method. The dense staining of a nuclear body in the young *æstivo-autumnal* parasite has often been noted, and in 1894 Okintschitz mentioned the fact that the nucleus of the young tertian parasite fails to stain by methylene blue.

(2) The tertian ring is usually coarse and irregular, but the *æstivo-autumnal* ring is geometrically circular, more delicate, with an extremely fine bow, and usually with a typical signet-like swelling.

(3) One or two grains of pigment are almost invariably found in the early tertian ring, but are, with nearly equal constancy, absent from the *æstivo-autumnal* signet-ring.

(4) My specimens confirm the statement of Gautier that the tertian ring is usually pigmented before the chromatin becomes subdivided, while the chromatin of the *æstivo-autumnal* ring is always subdivided before the appearance of pigment. In some cases, however, the chromatin of the tertian ring divides before the appearance of pigment.

(5) The infected cell is usually swollen from the moment of infection by the tertian spore, and commonly shrunken when harboring the *æstivo-autumnal* ring.

All of these characters are usually apparent in ordinary specimens, but naturally are most distinct in flatly spread and rapidly dried corpuscles. I have encountered no exception to these rules in cases of infection by the tertian parasite in New York, and cases of *æstivo-autumnal* infection from Cuba. In many of the irregular relapses in cases showing tertian organisms encountered among volunteer

soldiers during the winter of 1898-99, single ring-shaped parasites not admitting of positive identification have frequently been seen. The significance of this observation will be considered later (see section on Plurality of Species of Malarial Parasites, p. 490).

After a period of 6 or 8 hours the tertian ring is usually found to have developed an outgrowth which is actively amœboid in the fresh condition and appears in stained specimens as a tongue-like protrusion or turban-shaped mass attached to one segment of the ring (Plate XXIX, Figs. 4 and 5). The nuclear body meanwhile increases slightly in size, projecting into the ring, and the chromatin divides into several large granules.

At this period occurs the greatest amœboid activity of the parasite, and in some severe tertian infections the parasite may be found fixed in the height of its excursions, when it presents in stained specimens the peculiar appearance depicted in Plate XXIX, Figs. 9 and 10. Here the ring is unfolded and the body of the parasite is strung out into a number of slender threads with nodal thickenings. At times the number and delicacy of the threads greatly exceed those seen in the sketch, and in some red cells the parasite may be found distributed in a series of fine granules between which the connecting threads are with difficulty distinguished. These latter are probably to be classed with "quinine forms."

A close inspection of corpuscles harboring such parasites often discloses the presence in one corpuscle of two distinct nuclear bodies, indicating the co-existence of two parasites. Frequently in one corpuscle are found twin parasites, entirely separate one from the other, each of which shows a tendency to develop the long threads (Plate XXIX, Fig. 9). When, however, the threads are numerous and very thin it is usually impossible to find any break in their continuity, while in many instances the two parasites are distinctly united. The significance of these two forms will be considered later (p. 475 et seq.).

During the second quarter of the cycle the body and the nucleus of the parasite develop rapidly in size, amœboid motion and amœboid figures gradually diminish, and pigment is abundantly deposited in the form of fine dark brown or yellowish grains showing in the fresh

state active vibratory motion. The infected red cell continues to increase slightly in size, its hæmoglobin is progressively diminished, and granular degeneration is extreme. Depending upon the character of amœboid activity the variety of figures seen during this period is very great. Eventually, toward the end of 24 hours or possibly somewhat later, the parasite occupies three-quarters of the swollen cell, in the form of a spheroidal or elliptical, homogeneous body, the outer portion of which contains most of the pigment and is rather more deeply stainable than the zone immediately surrounding the nucleus (Plate XXIX, Figs. 12 and 13). The nucleus gradually increases in size, growing into the ring. It no longer has the appearance of a small highly refractive achromatic spot (after methylene blue), but takes a light bluish tinge with 1 per cent methylene blue, and stains less deeply than before with hæmatoxylin. At the end of this period the nucleus completely fills the ring, stains rather distinctly with methylene blue and sometimes exhibits a delicate bluish network.

By Nocht's method the changes in the nucleus are found to consist in the gradual subdivision of the chromatin granules, which finally become rather numerous, of minute size, and more difficult to stain. Usually these chromatin granules lie on the inner circumference of the bow of the ring, projecting within the ring, and partly surrounded by a "milky" unstained zone. This milky zone is often absent in young parasites in cells thinly spread and rapidly dried, but in older parasites it is always present.

Various other positions may be assumed by the chromatin mass, as follows: a subdivision of the granules into two distinct groups, separated by a strand of bluish stained protoplasm; an eccentric position entirely apart from the ring; a position midway between two rings formed in the same parasite; a position in the centre of the ring entirely apart from any bluish protoplasm; a circular arrangement about the periphery of the ring. Sometimes the smaller granules are grouped about a central larger granule, as has been noted in other protozoa whose nuclei are of the "intermediate type" (*Microglena*, *Euglena*).

The third quarter of the cycle is occupied by the continued growth of the parasite in the form of a large homogeneous richly pigmented body, which finally occupies at least four-fifths of the swollen red corpuscle, and by certain nuclear changes which it is difficult to follow in specimens stained by methylene blue or hæmatoxylin, but which are fully demonstrated by Nocht's method.

The exact limits within which the parasite may be termed "full grown" can be sharply fixed only with great difficulty, but there appears to be a period of at least 12 hours, during which there is little change in the structure of the organism and during which the body stains homogeneously and the nucleus occupies the entire ring. This period may be placed between the 24th and 40th hour of the cycle. A portion of it is occupied by nuclear changes belonging to the reproductive phase of the parasite's development.

After the appearance of a faint intranuclear network most authorities agree that the nucleus largely disappears, so far as can be determined in specimens stained by methylene blue or hæmatoxylin, and that it is next seen in the form of highly refractive achromatic spots in the meshes of the reticulated presegmenting body (methylene blue), and these again stain deeply with hæmatoxylin.

Nocht's method, however, fully demonstrates the nuclear changes which occur in the full-grown parasite. A considerable area, usually the entire original ring, is now occupied by a "milky" or slightly bluish staining substance in which lie a considerable number of very fine chromatin granules. These granules are usually difficult to stain, and being of very minute size they are difficult to see. This fact has led Ziemann and Gautier to admit the possibility that the chromatin may actually disappear at one stage of the development, especially since they have found some large parasites in which no chromatin was demonstrable. In my specimens there were a very few large tertian parasites in which no chromatin granules appeared, but these were not more numerous than younger forms which were also devoid of chromatin and must therefore be regarded as sterile. I therefore interpret the larger forms devoid of chromatin as sterile forms, and cannot accept the view that the chromatin entirely dis-

appears at any stage of the fertile parasite, a view which is at variance with biological principles.

After the subdivision of the chromatin has reached a limit the next change, observed in a considerable number of parasites, appears to consist in the extension of a portion of the milky substance and its chromatin granules into the body of the parasite (Plate XXIX, Fig. 13.) At the same time the granules of chromatin increase in number. Other forms may be seen in which the "milky substance" and chromatin granules occupy an elongated space within the body of the parasite, and in such cases the beginning concentration of pigment and deeper stain of the parasite indicate the presence of the presegmenting stage (Plate XXIX, Fig. 14).

Presegmenting Bodies.—In specimens stained by methylene blue the first demonstrable indications of the division of the parasite are seen in *deeper staining capacity* and *tendency toward reticulation* which appear throughout the whole or in a part of the body of the parasite. Occasionally these changes may be noted in one-half the parasite while the other half retains the homogeneous appearance of the "full-grown" organism. Usually the process is found to have affected the entire organism, giving the very characteristic forms sketched in Plate XXIX, Figs. 15 and 16. In the presegmenting bodies the pigment is gathered in a reduced number of coarse grains, which lie in the body of the parasite in a position determined by that of the new multiple nuclei.

These bodies were first described by Golgi in fresh blood and properly interpreted as belonging to the process of segmentation. Later they were described by Marchiafava and Celli³ as vacuolated parasites, the highly refractive nuclear bodies appearing in the fresh condition very much like vacuoles. Still later, Celli and Guarnieri sketched them from specimens stained in the fresh condition, regarding some as showing partial segmentation, others as vacuolated parasites, although they accurately described the appearance of the nuclear particles invariably found within these "vacuoles," while still others they supposed to be groups of confluent parasites. *i. e.* true plasmodia. Mannaberg's descriptions (1899), referring only to fresh blood, do not include these bodies, nor

³ *Atti de R. Accad. med. di Roma*, 1887, 2. S., iii, p. 277.

have they found a distinct place in his plates, although some of the figures in his Plate IV indicate that they have not escaped his observation.

Thayer and Hewetson,⁴ in their careful study of the parasite in fresh blood, designate as presegmenting bodies the parasites with collected masses of pigment (*corpi con blocchetto* of the Italian writers).

Laveran⁵ refers to the similarity in appearance between a nuclear body and a vacuole, but he neither describes nor depicts the presegmenting reticulated parasite.

Ziemann⁶ describes the presegmenting bodies as they appear after his or Romanowsky's staining methods, but the plates would not enable one unfamiliar with the subject to identify these forms in specimens stained by eosin and methylene blue.

The reticulated presegmenting tertian parasites were seen in every case of the present series examined within the 6 or 8 hours preceding the chill, and often in belated parasites shortly after the chill. Many transitional stages between the homogeneous adult parasite and the perfect rosette may be seen in rich infections. They are well demonstrated by eosin and methylene blue, especially as regards the increasing density of stain and the reticulation. After hæmatoxylin the multiple nuclei stain deeply by methylene blue.

By Nocht's method a series of interesting nuclear changes may be followed in the presegmenting forms. After the mass of enlarging chromatin granules and milky substance has flowed out into the elongated form described above, the chromatin granules leave the central clear space and make their way in groups out into the body of the parasite. Various stages of this process may be followed in specimens taken at suitable periods, and some observed phases are seen in Plate XXIX, Figs. 13-16. Considerable difference in the numbers of such groups may be noted in different cases. Usually a large number of ill-defined groups are seen, before the central mass of granules is exhausted (Plate XXX, Fig. 14). In one specimen the compact nuclei of the young spores appeared to form in one segment of the parasite before the main mass of granules had become exhausted.

⁴ *The Johns Hopkins Hospital Reports*, 1895, v, p. 3.

⁵ *Traité du paludisme*. Paris, 1898, p. 62.

⁶ *Ueber Malaria- und andere Blutparasiten*. Jena, 1898.

Each of the groups appears always to be surrounded by a milky zone, and the mass of granules is often of a peculiar triangular form (Plate XXIX, Fig. 12). During these changes the pigment granules increase in size, diminish in number, and are distributed in the meshes of the now distinctly reticulated body of the parasite.

Tertian rosettes (Plate XXIX, Fig. 17) are usually seen in the circulation three or four hours before the chill, most abundantly just before the chill, and a few are often to be found for one hour or more after the chill. These limits may occasionally be much wider, as Marchiafava and Celli have seen rosettes 2 to 6 hours before the chill, and 6 to 7 hours thereafter; and indeed, when the different broods of parasites are not very distinct, there is no reason why occasional rosettes should not be found at any period of the main cycle.

Of the three types of sporulation described by Golgi, the second, according to which the entire parasite is divided into spores, leaving nothing but pigment, is undoubtedly the usual process. As regards Golgi's first type, in which only the peripheral portion of the parasite divides, leaving a distinct central globular pigmented body, most stained specimens fail to show convincing evidence that the physiological process of segmentation may be subject to such an important modification, nor does it appear in recent literature that the existence of this variety of segmentation has been fully verified. In a few specimens from patients taking quinine, I have seen rare segmenting bodies which resemble those described as above by Golgi, but never in fresh cases.

Golgi's third type of "partial segmentation," together with the "lateral circumscribed sporulation" of Celli and Guarnieri, may frequently be seen in rich tertian infections in fresh blood, but according to the evidence of stained specimens this must be classed with the presegmenting forms.

The tertian rosette is usually distinguished by its large size and considerable number of spores—fifteen to twenty. In the present cases it did not appear, however, that the identification of the tertian rosette could always be based upon the number of spores. Marchiafava and Bignami have described tertian rosettes with 40 to 50 spores.

With the smaller number of spores the rosette was usually much larger than either the quartan or the æstivo-autumnal body.

The nuclear changes demonstrated by Nocht's method in the tertian rosette consist principally in the gradual fusion of the new-formed groups of chromatin granules into single compact globules, which are partly surrounded by "milky zones." While the rosette is still compact the vesicular shape of the spore is distinct. The outer segment of the ring is usually thickened, the nucleus tends to lie near the inner pole, and between the nucleus and outer segment is a small "milky zone." The pigment is usually collected into a central block or mass of granules, but may be found variously scattered among the spores, or along the periphery of the rosette.

The Quartan Parasite.

The earliest form of the quartan parasite (Plate XXXI, Fig. 1), as seen in the stained red cell, is practically indistinguishable from that of the tertian organism, but its true character may usually be suspected from the slightly shrunken appearance of the infected cell. In fresh specimens the higher refractive quality of this parasite is often, however, sufficiently characteristic for its identification. After a very slight increase in size the quartan parasite becomes rather easy to distinguish in both fresh and stained specimens, for it usually remains smaller, more compact, and is more richly and coarsely pigmented than the tertian organism. As with the latter, the nuclear body is found projecting into the ring (Plate XXXI, Figs. 1-4). In fresh specimens at this period, the higher refractive qualities and slower amœboid motion are additional diagnostic characters.

The growth of the quartan ring is very similar in all important respects to that of the tertian, while its distinguishing features, especially the abundance of coarse pigment grains, are uniformly retained (Plate XXXI, Figs. 5-8).

During the presegmenting stage the characters of the quartan parasite are markedly different from those of the tertian. On account of the slower progress of sporulation, and from the greater tendency of the quartan parasite to complete its cycle in the general circulation, quartan presegmenting bodies are relatively much more numerous

in the stained specimens than are the similar forms of the tertian organism. In some specimens taken several hours before the chill the majority of organisms found may present the markedly reticulated structure indicative of approaching division. The multiple nuclei are less numerous, and the pigment is more abundant, and is often found in irregular, partly radiating rows. The coarsely reticulated, relatively small, and richly pigmented bodies lying in markedly shrunken cells are very characteristic and not readily confused with any other form of malarial parasite commonly found in the peripheral blood (Plate XXXI, Figs. 10-12). In some severe æstivo-autumnal infections, showing many parasites of all stages in the peripheral blood, somewhat similar spheroidal or presegmenting forms of the same general appearance may be found in considerable numbers, but as will be seen by reference to Plate XXX, the character of the pigment in the æstivo-autumnal parasite is very different. Such cases are very rare, and readily recognized on clinical grounds, being almost invariably of the pernicious type.

The quartan segmenting bodies are relatively more abundant in the peripheral circulation than are rosettes of any other variety of malarial parasite, and are easily identified by the small number (6 to 12) and comparatively large size and geometrical arrangement of the spores (Plate XXXI, Fig. 14).

The Æstivo-Autumnal Parasite.

The following description applies to a group of organisms which, according to the Italian school, comprises two or three varieties of malarial parasites. Waiving for the present the question of plurality of species, the entire group will be described as one, and the grounds alleged for their separation will be considered later. The present description rests upon the examination of some 260 cases of æstivo-autumnal infection occurring in U. S. soldiers who had shortly before arrived at Montauk Point from Cuba, and on a smaller number of cases seen in New York during the past few years. The conclusions drawn from the original examinations have been verified or modified by more careful study of these specimens during the past winter and also by the microscopical study of the tissues of a number of cases coming to autopsy.

The *earliest form* of the aëstivo-autumnal parasite seen in the red cells, in the present cases, was very similar to that of the tertian and quartan parasites, but was slightly smaller than either, and was often distinguishable from the tertian by the shrinkage of the cell, and from the quartan by its distinctly smaller dimensions (Plate XXX, Figs. 1 and 2). In fresh specimens the young amœboid body usually showed a low refractive index as compared with the tertian and quartan parasites. It was never pigmented. Associated with the intracellular spores there were frequently seen in the plasma small spheroidal bodies, exhibiting an active rolling motion and occasional blunt projecting points (pseudopodia?), which, on becoming arrested by contact with red cells, were found to be indistinguishable from the intracellular bodies. Although seldom identified in stained specimens, it appears probable that these bodies were young extra-cellular forms of the parasite. The positive identification of these extra-cellular bodies, however, appears to be a very hazardous undertaking (cf. Ziemann, p. 49). In dried specimens stained by Nocht's method, however, the young extra-cellular parasite may be positively identified from the presence of a characteristic mass of chromatin. In my specimens such extra-cellular bodies were rarely encountered.

At a very early period of its development the aëstivo-autumnal parasite in the present cases assumed a very characteristic *ring shape*. Many of these rings early developed a thickening of one segment, and to these bodies of various sizes the term "signet-ring" very aptly applies (Plate XXX, Figs. 5-7). It was noted that in some cases the rings failed to exhibit this thickening, but remained of a *uniform but very fine caliber throughout* (Plate XXX, Fig. 4). The period during which the rings retained this uniform caliber was not determined, but bodies of this type were seen measuring at least 3 μ in diameter. They nearly always presented two nuclear bodies, lying at opposite poles or close together. Occasionally such rings were found to have unfolded and to be stretched like a thread across the cell, the nuclei appearing at inconstant intervals. In other cases no rings of this type were seen, all showing the thickening of the signet and a single nuclear body. In the majority of cases rings of both types were associated in variable numbers.

No connection was demonstrated between the clinical features of these cases and the occurrence of these two types of rings, as both were found in cases showing intermittent tertian paroxysms, quotidian paroxysms, remittent fever of seven days' duration, and irregular fever for longer periods.

Multiple infection with the young rings was very common in the red cells of most cases of the present series and, as a rule, its occurrence was proportionate to the severity of the disease. In the peripheral blood three parasites were often found in the same red cell, occasionally as many as four; while in smears of the marrow of a fatal case infection of one red cell with four rings was common, five parasites were occasionally seen in the same cell, and one slightly swollen red cell was encountered containing seven well-formed rings (Plate XXX, Fig. 2). These observations accord with those of Ziemann (op. cit., p. 49), who found often three and four parasites, and once as many as five, in one red cell.

It appears in the descriptions of *Hæmamoeba immaculata*, which is said to sporulate without producing pigment, that most of the rosettes contain comparatively few spores, averaging from 6 to 10 (Marchiafava, Bignami, Ziemann, Marchoux, Grassi and Feletti). The close resemblance to a non-pigmented rosette presented by some of these red cells harboring 5 or 6 young parasites was very striking. In my specimens (Plate XXX, Fig. 2) there could be no doubt as to the proper interpretation of the appearances.

Multiple infection of the red cell appears in rather rare instances to lead to the development of a peculiar form of the young æstivo-autumnal parasite on which Mannaberg bases his unique theory of the development of crescents. This body consists in the apparent union of two rings by a fusion of their nuclear bodies (Plate XXX, Fig. 3). Mannaberg depicts all transitional forms between these bodies and the fully developed crescent. Many examples of these double rings were encountered, but the various transitional forms from young double rings to crescents, shown in Mannaberg's plates, were not seen in the present cases.

The signet-ring forms frequently reached a diameter of 4 μ , while

still retaining the peculiar thickening of one segment and a very distinct nuclear body staining with methylene blue and surrounded by a narrow achromatic zone (Plate XXX, Figs. 6 and 7). Beyond this size, when persisting in the peripheral blood, the growth of the parasite produced an irregular body in which the outline of the ring became more or less obscure. The exact periods required in the development of these rings were not definitely determined, but in six cases taking quinine typical signet-ring forms were seen in the peripheral blood 60 to 72 hours after the beginning of the paroxysm. It seems probable, however, that these were belated individuals belonging to a somewhat scattered brood of which the majority either had retired to internal capillaries or had been destroyed by quinine. Yet in some cases in which the blood changes were followed at intervals of 6 to 12 hours, the increase in the size of the rings proved to be surprisingly slow, and the impression was obtained from these cases that the full development of the signet ring usually requires at least 24 hours and sometimes longer.

In the majority of cases the ring forms seen in the peripheral blood failed to show any trace of pigment, especially in patients showing distinctly intermittent quotidian or tertian paroxysms. In a considerable number of instances, however, especially in very severe and fatal infections, the largest rings exhibited a few very minute pigment grains, and were then usually associated with older pigmented forms.

The *later forms* of the æstivo-autumnal parasite are rather rarely seen in the peripheral circulation. Most of the Italian writers speak of their occurrence in the blood of the finger as being very unusual but not unknown. Sacharoff, in two cases of æstivo-autumnal infection, saw many rosettes in the peripheral blood. Ziemann reports that in malignant tertian cases occurring in Italy, he could follow, in the blood, the complete cycle, but that in cases occurring in Kamerun the later forms were not found in the finger blood. Plehn describes a variety of parasite which he believes is peculiar to hæmoglobinuric fever, and of which the later forms are of very small size but abundantly represented in the peripheral blood.

In five cases of the present series the entire developmental cycle of the æstivo-autumnal parasite could be followed in the peripheral

blood, and on the forms observed in these cases is based the present description of the later phases of this parasite.

After the ring has reached its full size (4 μ , 24 hrs. +), the swollen segment begins to increase in bulk and to involve a larger portion of the circumference, yielding forms seen in Plate XXX, Figs. 8-10. Some of these forms closely resembled the turban-shaped rings of the tertian parasite (Plate XXIX, Figs. 4 and 5), but were much smaller. A few fine pigment grains were usually found scattered along the periphery of the growing segment. Forms corresponding to the full-grown tertian parasite with homogeneous body were rarely seen in the peripheral blood of these five cases, but occasionally some were encountered (Plate XXX, Figs. 11 and 12) occupying three-fourths of the shrunken cell, staining homogeneously with methylene blue, and failing to exhibit a distinct nuclear body after methylene blue or hæmatoxylin. In sections of tissues from fatal cases these bodies appeared to be rather more numerous, but it was very difficult to distinguish in sections such homogeneous bodies from the slightly reticulated bodies representing the next stage of development. The fact that all other stages of the parasite were abundantly represented in the blood of these cases, while the homogeneous forms were very few, indicates that this period of development passes rapidly with the æstivo-autumnal organism.

Most of the larger forms of the parasite seen in the blood-smears of these five cases gave evidence of approaching segmentation, exhibiting a distinctly reticular structure and a condensation of pigment into one or two clumps (Plate XXX, Figs. 13 and 14). In many of these bodies the original ring persisted at one segment of the parasite, but appeared to be of reduced size and was sometimes subdivided by strands of protoplasm. The nuclear body at this period failed entirely to stain with methylene blue and was indistinct after hæmatoxylin, resembling in this respect the full-grown homogeneous tertian organism. The presence of a distinct achromatic spot adjoining the clump of pigment was very frequent in these forms and this spot was found by Nocht's stain to be composed of chromatin granules. The reticular structure of these bodies was usually distinct and the meshes were coarse.

The further development of the presegmenting forms is represented in Figs. 14 and 15, Plate XXX. In them the reticular structure becomes more distinct, the pigment is still further concentrated, and the subdivided nuclear bodies appear as small achromatic spots in the meshes of the reticulum and again stain distinctly with hæmatoxylin.

Rosettes (Plate XXX, Fig. 16) appeared in the peripheral blood of the five cases in moderate numbers and exhibited, in all, a very uniform structure. The pigment was grouped in a central granular clump, or, rarely, was somewhat scattered. The spores seemed to be arranged in two rows, but this appearance was probably an optical effect produced by the flattening of the more or less spheroidal body of the rosette, the spores originally lying in the central axis of the parasite falling, in the hardening process, within those lying in the periphery. When admitting of accurate enumeration their numbers were found to vary between 18 and 21. The same number of spores was repeatedly counted, in favorable specimens, smeared from the marrow of fatal cases. In sections of the tissue of fatal cases, however, the number of spores appeared to vary between wider limits, *i. e.*, 8 to 20, but as the entire rosette need not always be included in the section, the observations made in smears are the more reliable. A rim of hæmoglobin invariably surrounded the rosette and strands of hæmoglobin were frequently found running between the spores for a variable distance, sometimes within the outer row. These rosettes differed from tertian segmenting forms in the smaller size of the body and shrunken appearance of the cells, and in the small size, *but not in the number*, of the spores.

In none of the blood-smears nor in sections of tissues of the fatal cases were any rosettes seen without pigment. Although the arrangement of the spores and pigment often varied, there were no indications of the subdivision of the process of segmentation into the three types described by Golgi, nor were any forms seen which resembled the bodies described by Celli and Guarnieri,⁷ and referred by them, with some uncertainty, to irregular sporulation.

The changes in the chromatin of the æstivo-autumnal parasite can be

⁷ *Fortschritte d. Medizin*, 1889, vii, p. 528.

followed in specimens stained by Nocht's method (Plate XXX, Figs. 6-12), but on account of the smaller size of the parasite and the scarcity of older forms in the blood, it is difficult to trace the early phases of segmentation. In the young ring forms the early subdivision of the chromatin has been noted by Gautier, and in my specimens was a prominent differential character from the tertian rings. A great variety of appearances was produced by the irregular subdivision and distribution of the chromatin in the young *æstivo-autumnal* parasite, many of which have been sketched or described for the tertian rings. The grains were usually quite small and were sometimes apparently fused into a spindle-shaped mass, lying within the ring. Other peculiarities noted were: a markedly unequal size of the grains, a widely separate position, a frequent concentration in the centre of the ring, and, very rarely, a complete absence of chromatin.

After 24 hours' growth, the chromatin granules became more numerous and extremely minute, and were enclosed in a trace of the "milky substance," as in the tertian parasite.

When any considerable quantity of pigment gathers in the *æstivo-autumnal* parasite it is usually found in one or two groups, but rarely is diffuse. When the parasite has reached the full-grown homogeneous stage the pigment is commonly found concentrated in a single compact mass. This early concentration of pigment is one of the chief features which distinguish the *æstivo-autumnal* from the tertian parasite in the presegmenting stage. This fact has been fully emphasized by Gautier and was very uniformly illustrated in my cases. The changes in the chromatin in the presegmenting *æstivo-autumnal* body (Plate XXX, Figs. 13-15) are similar to those of the tertian parasite. In some of the specimens the chromatin granules were found in radiating lines stretching from the parent mass to the new peripheral groups (Plate XXX, Fig. 13). In many specimens the peripheral group of granules was well formed while the central portions of the body contained many diffuse granules (Plate XXX, Fig. 14). The relative quantity of chromatin in some of these bodies appeared surprisingly large. The spores in the mature rosettes usually contained single compact grains of chromatin (Plate XXX, Fig. 16), which stain readily by methylene blue, but in some rosettes

two large granules of chromatin were seen in a few spores, although the rosette seemed ready to burst. The double nuclei seen in many young æstivo-autumnal rings may perhaps be referred to the incomplete fusion of the chromatin in the rosette.

III. ON THE PLURALITY OF SPECIES IN THE ÆSTIVO-AUTUMNAL GROUP OF PARASITES.

The probability that several species of parasites are concerned in the severe types of malarial fever prevailing in warm climates, especially during the summer and autumn, has been maintained chiefly by Marchiafava, Celli, Bignami, and Grassi. From their studies of this group of fevers they divide the æstivo-autumnal group of parasites into two species: (1) The quotidian, and (2) the malignant tertian.

1. *The quotidian parasite*.—The typical fever-curve of this variety the authors found rather rarely, more frequently in relapses than in initial seizures, while a postponement of paroxysms was usually observed, and a continuous fever was very common. The typical attack is short, the fever lasting 6 to 8, rarely 12, hours, the temperature then falling to 37° C. or lower. The descriptions of the morphology of this parasite unfortunately refer only to the appearances in fresh blood.

During the rise of the temperature, the sweating stage, and the first hours of apyrexia, the blood was found to contain a variable number of red corpuscles infected with one or more very actively motile, or non-motile, parasites of discoidal or ring shape. During the afrebrile period the parasite increased in size, the amœboid motion diminished or ceased, and fine pigment grains were deposited along the periphery of the organism. Later, in the larger forms, the pigment gathered in a single clump or heap of grains. During the entire development the infected red cell diminished in size and presented a "brassy" color as a result of "acute necrosis" induced by the parasite. Rosettes were seldom encountered in the blood of the finger, segmentation occurring principally in the internal organs, as seen in the aspirated splenic blood. Rarely segmentation occurred before pigmentation, but usually the numerous round or oval spores were found grouped about a central pigment mass, the rosettes being much smaller than those of the quartan or common tertian parasite. Contrary to the rule in malignant tertian infections, the young parasites were found in the blood from the beginning of the paroxysm, and, except in very mild cases, there was no

period in the cycle when the parasites were absent from the blood of the finger.

2. *The malignant tertian parasite* is distinguished by the authors on both clinical and morphological grounds. Clinically the typical paroxysm begins with a sharp elevation to about 40° C., the febrile period lasts 24 or 36 to 40 hours, is marked by a pseudo-crisis and pre-critical elevation, the fever describing, in the three-hourly chart, a characteristic course which differs from that of the mild or common tertian paroxysm. A tendency towards various irregularities is common.

In the blood the parasites may be scarce or even entirely absent at the beginning of the paroxysm. At the height of the fever the red cells contain certain small, non-motile, annular or disk-shaped bodies, or irregular amœboid bodies, which begin to show pigmentation towards the approach of the afebrile period. Most of the parasites then disappear from the peripheral blood, and rosettes are rarely seen except in some very rich infections. The presegmenting forms are round or ovoid, are one-fourth to one-half the size of the red cell, and the pigment is gathered in a single clump or in a mass of vibrating granules. The rosettes occupy about two-thirds of the red cell and exhibit two rows of spores which usually number 10 to 12, rarely 15 to 16. The infected cells are markedly shrunk and present a "brassy" or "golden" appearance.

The authors distinguish the malignant tertian parasite from the common or mild variety on the following features:

(1) The malignant tertian parasite is smaller in all stages. (2) It assumes the ring shape, which, in the benign tertian parasite, is never seen. (This statement has been shown by many writers to be erroneous. Most of the young, mild tertian parasites are ring-shaped.) (3) Its pigment is less abundant and often non-motile, while in the other the pigment is very abundant and always in vibratory motion. (4) The rosettes are smaller, contain only 10 to 12 (rarely 16) spores (?), and are rarely seen in the finger blood. (5) The infected cell is shrunk instead of being swollen, as with the mild tertian infection.

From the quotidian parasite the malignant tertian is distinguished on the following grounds:

(1) The malignant tertian amœba is, in corresponding stages, larger and less transparent than the quotidian. (2) In the tertian parasite the amœboid movement is livelier, so that the resting discoidal forms are less frequent than with the quotidian parasite. The larger pigmented tertian forms also are often amœboid, this property persisting for 24 hours or longer. (3) The pigment of the tertian parasite is often

vibratory, but never in the quotidian. (4) In the quotidian rosettes pigment is sometimes wanting. (5) The appearance in the finger blood of a new generation of tertian parasites is seen some hours after the beginning of the paroxysm, therefore much later than with the quotidian infection.

Marchiafava and Bignami admit that the similarity between the malignant tertian and the quotidian parasites is very great, and that the differential diagnosis is very difficult, and possible only from the full-grown forms seen just before the paroxysm. They apparently do not feel quite certain that the quotidian and malignant tertian parasites are separate species, as is indicated by the following extract from their discussion on this point:⁸

"The remarkable points of resemblance between the quotidian and malignant tertian parasites make it very difficult to solve the question whether we have to do with different sorts of parasites in the strict sense, or with one and the same parasite which varies greatly in the time of its development—24 to 48 hours—and there are all intermediate degrees. On this latter theory it becomes easy to ascribe the morphological differences to the varying length of the cycle. But various facts oppose this hypothesis. First, the clinical types of the quotidian and tertian are clearly distinct from each other, and have a certain stability which is maintained in relapses and recurrences. Second, we have never met with intermediate forms or transitional cases, although it is very difficult to interpret the irregular fever. Granting that the question cannot at present be solved definitely, . . . we are inclined to adopt the view that the amœba of the quotidian and the amœba of the summer tertian are closely related varieties of one and the same parasite."

Mannaberg⁹ accepts the views of Marchiafava, Celli, and Bignami, in respect to the separate nature of the malignant tertian and a quotidian group of parasites, and his description of the morphology of the parasites does not differ from that of the preceding authors whom he largely quotes. From his description of the single case of quotidian fever it is impossible to determine how many groups of parasites were present in the blood.

Grassi and Feletti¹⁰ include all tertian parasites in one class (*Hæmamaeba vivax*) and state that an easily recognized species of malignant quotidian parasite (*H. pravox*) is found in Catania during the sum-

⁸ Translation, *The New Sydenham Society*, London, 1894, vol. cl.

⁹ *Die Malariakrankheiten*. Wien, 1899.

¹⁰ *Centralbl. f. Bakter.*, 1891, x, pp. 449, 481 and 517.

mer and autumn. Their opinion appears to be based largely on an acceptance of the views of Marchiafava, Celli, and Bignami, as they offer no detailed description of these parasites nor of the cases in which they have found the latter variety.

Of the cases reported by Thayer and Hewetson, in 114 young æstivo-autumnal parasites were seen in the blood, and of these cases 73 exhibited quotidian fever. Although the examination of these cases was made almost wholly with fresh blood, it is significant that they found no evidence on which to subdivide the group of æstivo-autumnal parasites.

Ziemann, from the study of 210 cases of æstivo-autumnal infection, was unable to find sufficient grounds for the subdivision of this group. He, however, mentions the fact that the small parasite observed in cases occurring in Crema and Grosseto showed slightly less amœboid motion than that found in cases in Kamerun, and admits the possibility that quotidian æstivo-autumnal fever may be referable to a parasite which completes its development in 24 hours. He regards it as equally probable, on the other hand, that the quotidian fever is caused by the growth of two generations of small tertian parasites, but in his entire series he was unable, on account of the disappearance of the parasites from the peripheral blood, to determine accurately the length of the cycle. In his cases of malignant tertian fever, the prolongation of the febrile paroxysm, described by Marchiafava and Bignami as a characteristic feature of the malignant tertian infection, was not always to be seen. On these various grounds he concludes that all forms of the æstivo-autumnal parasite belong to one group of which the cycle varies in length from 24 to 48, or possibly 72 hours.

Gautier,¹¹ who has very carefully studied the malarial parasites of the Caucasus in specimens stained by Romanowsky's method, has failed to find any which he could regard as completing their cycle in 24 hours. Gautier's charts illustrating the forms of the parasite prevailing in the blood at various periods of the cycle very graphically illustrate the difficulty of following the development of the æstivo-autumnal parasite in the peripheral blood. The prolongation of the febrile paroxysm was sometimes present, sometimes wanting, and appeared to be referable to the maturation of sub-groups of parasites (see his Curve III).

In my cases there was a moderate number of pure tertian paroxysms caused by infection with a parasite morphologically identical

¹¹ Ueber den Parasit Laveran etc. (Russian). Moscow, 1896, and *Ztschr. f. Hyg.* 1898, xxviii, p. 439.

with the malignant tertian of Marchiafava and Bignami. The prolongation of the paroxysm and the pseudo-crisis were sometimes observed. I encountered the same difficulty experienced by Ziemann and Gautier in determining the length of the cycle from the parasites in the peripheral blood, and believe that it is seldom possible on this evidence alone to demonstrate a 48-hour cycle for the parasite. The infecting brood is seldom very compact, and in rich infections, in which alone presegmenting bodies and rosettes appear in the blood, the groups are nearly always multiple. In my preliminary report¹² on the Montauk cases this difficulty was noted. Indeed, in 6 cases, it seemed impossible to find any marked change in the size of the parasites in the peripheral blood for three days after the chill, the ring forms persisting for that period and being constantly supplied by constantly maturing rosettes in the visceral capillaries (see also Gautier's tables). The frequency of cases showing the ring forms persisting, with slight changes, for two or three days, led to the belief that the cycle of development must sometimes extend over 48 hours. I was not then familiar with Golgi's conclusions, but since find that this observer, who is certainly qualified to know what evidence is needed to establish a 48-hour cycle, concluded that "the parasites in the blood of æstivo-autumnal cases are only an index of the infection, have little to do with the real pathogenesis of the fever, and that they represent early phases of a cycle which is much longer than has been believed."

In one of my cases, however, in which the examinations of the blood were supplemented by microscopical examination of the viscera, a 48-hour cycle appeared to be demonstrated. For the present purpose, therefore, the temperature chart furnishes by far the most convincing evidence.

The majority of my cases, however, showed quotidian excursions, and the temperature chart was of little value in determining the length of the cycle. In these the parasites in the blood, and occasionally in the viscera, were submitted to microscopic examination in the fresh condition, and in specimens stained by eosin and methylene blue, and by Nocht's method. From this study, only one feature was noted

¹² *New York Medical Journal*, 1899, lxix, pp. 114. and 149.

which could possibly serve to separate the parasites into two groups, and this related to the form of the young rings. The majority of the young æstivo-autumnal rings exhibit a single chromatin granule and a distinct thickening of one segment of the ring, and this latter character is maintained from the very smallest form up to bodies at least $4\ \mu$ in diameter. In addition to these signet-ring forms, other rings were seen which lacked the signet, were provided with two chromatin granules usually located at opposite points in the circumference of the ring, and which retained this appearance up to a considerable size ($3\ \mu$, rarely $4\ \mu$). Such rings have been repeatedly observed before and accurately sketched (Gautier, Ziemann, Marchoux). See Plate XXX, Fig. 4.

In some severe cases these rings, lacking the signet, constituted the majority of all those seen in the blood; usually both forms were abundantly present, and in some distinctly tertian cases, while the signet-rings were more numerous, the other type was also represented in small numbers. I am, therefore, unable to conclude that these peculiar rings belong to a separate species of parasite with a short cycle of development.

No other morphological differences were noted in the young rings of these cases. It is possible, but hardly probable, that the quotidian parasite, if it exists, was not represented among the cases seen at Montauk.

Between parasites of larger size, as seen in the blood and in smears of the viscera of fatal cases, considerable difference in size was noted, but the smallest presegmenting bodies and rosettes encountered were found associated only with the usual form of signet rings. My observations on the parasites in the fresh condition failed to show any uniform difference in the refractive quality or amœboid activity, but under the circumstances they could not be pursued so extensively as was desired.

The evidence secured failed therefore to establish any clinical or morphological grounds on which to separate the parasites of pernicious malarial fever into two or more groups.

It does not seem likely that such a division can be successfully maintained except on morphological grounds. The difficulty in fol-

lowing the development in the blood of even a 48-hour parasite is well illustrated by Gautier's tables, and must be considerably increased when dealing with a more rapidly maturing form. The tendency of the æstivo-autumnal parasite to be held in the viscera during its later phases very greatly complicates the undertaking. In the cases of quotidian infection reported in detail by Marchiafava, Bignami, and Guarnieri, one fails to find, in the results of the blood examination, convincing evidence of the existence of one group of parasites with a 24-hour cycle. The clinical peculiarities observed in these cases are not without significance, but are of themselves entirely inconclusive and still require confirmation. The evidence on which the quartan, tertian, and æstivo-autumnal parasites are separated is of entirely different value from that on which it is proposed to divide the æstivo-autumnal group. Distinct morphological characters have not been clearly established, peculiar clinical features have not been shown to characterize any considerable group of cases, the developmental phases of a 24-hour cycle have not been demonstrated in the blood or viscera, and it would seem that further observations are required before the existence of a quotidian parasite can be accepted even as a working hypothesis.

The later position of Marchiafava and Bignami, admitting that the existence of two species in the æstivo-autumnal group is not proven, would therefore seem to be justified.

That 72 hours may occasionally be required for the cycle of the æstivo-autumnal parasite is indicated by the observation of a few cases of quartan fever with this infection. Such cases have been reported by Gautier and Ziemann. The paroxysms, in Ziemann's case, were twice repeated, with intervals of two days; the fever almost completely subsided in the interim; and there seems to be no reason to suppose that the irregular maturation of tertian broods could possibly have produced the paroxysms.¹³

¹³R. Koch, whose report on malaria in tropical countries has appeared since the completion of this article, has also reached the conclusion that there is but one species of the æstivo-autumnal parasite, and that in fresh cases the fever is uniformly of the tertian type, but later tends to become more and more irregular. He considers "tropical" a more appropriate epithet than "æstivo-autumnal" to designate this parasite and the fever caused by it (*Deutsche med. Wochenschr.*, 1900, p. 781).

Hamamæba immaculata.

Grassi and Feletti, Marchiafava and Celli, Bignami, Guarnieri, Sacharoff, Marchoux, and Ziemann, report cases in which rosettes free from pigment were found in the blood or viscera. Most of these authors, while admitting that the parasite may occasionally sporulate without producing pigment, are not inclined to regard *Hamamæba immaculata* (Grassi) as a separate species.

Grassi and Feletti, however, claim to have observed in a bird pure infection with a variety of parasite which failed to produce pigment, and regard the appearance in the human subject of rosettes without pigment as evidence of infection by a distinct species of parasite. Manaberg also accepts this view.

In the report of the examination of the viscera of this bird by Grassi and Feletti no mention is made of the presence or absence of pigment, and it is impossible to determine whether or not the infection had failed to produce pigment in the viscera as well as in the peripheral blood. In all cases in which pigment-free rosettes have been found in the blood of human subjects there have been found the usual pigment deposits and pigmented rosettes in the viscera. That there is considerable variation in the quantity of pigment produced by the parasite in fatal cases is shown by the reports by Marchiafava and Bignami of fatal cases in which a microscopic examination was required to show the presence of very scanty deposits in the viscera. Ziemann mentions in this connection that he has seen a presegmenting body of the benign tertian type which was entirely free from pigment.

I have already mentioned (p. 447) the marked resemblance which red cells, harboring five or six parasites, may bear to pigment-free rosettes. Most of these rosettes, as described, contained a small number of spores (6 to 10). In the sketches of Marchiafava and Celli, as noted by the authors, the spores of the pigment-free rosettes are of unusually large size. Their appearance is almost identical with the cell harboring seven young parasites sketched in Plate XXX, Fig. 2, from the marrow smears of a fatal case. If the latter cell had been found in a section of tissue it would have been scarcely possible to distinguish it from a rosette without pigment.

In a drawing accompanying the article of Bastianelli and Bignami,¹⁴ is a nearly normal red cell apparently infected with six young parasites, in explanation of which the authors suggest an irregular form of segmentation. If the drawing is accurate, it appears to me that the cell

¹⁴ *Bull. d. r. Accad. med. di Roma*, 1894, xx, p. 151, plate i, fig. 26.

is too little altered to have long harbored a growing parasite, and that the young parasites are too large for spores. The drawings of the cerebral capillaries, filled with rosettes without pigment, hardly admit, however, of this interpretation. Nevertheless, a failure of the human malarial parasite to produce pigment is such a violent departure from its ordinary physiology that the fact should rest only on the most absolute proof, and it may not be amiss to have suggested a possible source of error in this field.

In any case the grounds are insufficient to warrant the classification of *Haemamæba immaculata* as a separate species of parasite, and seem at best merely to justify the opinion of other observers that pigment-free rosettes, as seen in the human subject, are an occasional form of the æstivo-autumnal parasite. This opinion is well set forth in the words of Marchiafava and Bignami:¹⁵ "We cannot allow that a distinction should be drawn between the *Haemamæba præcox* (tertian parasite of Grassi) and the *Haemamæba immaculata*, as two separate species. Segmentation with no pigmentation has been observed by Marchiafava and Celli, but only in very rare cases, and always together with pigmented rosettes. So that in these cases it would be necessary to suppose a double infection, an hypothesis that is devoid of all foundation. We shall feel unable to change our opinion until we meet with cases which show no trace of melanæmia and which, therefore, mean a pure infection with the *Haemamæba immaculata*."

IV. THE NUCLEAR BODY OF THE MALARIAL PARASITE.

From the earliest period of the minute study of the malarial parasite certain structures in its body have been recognized as probable nuclear elements, but the exact significance of these structures and their relation to the definite nuclear elements of metazoan cells have never been fully determined.

In 1889, Celli and Guarnieri,¹⁶ from the examination of fresh malarial blood stained by methylene blue in ascitic fluid, described in the larger parasites, an outer deeply staining ectoplasm and an inner nearly achromatic endoplasm. In the lightly colored endoplasm, surrounded by a narrow, perfectly achromatic zone, was a sharply marked body of variable structure, sometimes compact, sometimes reticulated, but evi-

¹⁵ New Sydenham Society's Translation, op. cit.

¹⁶ References to the authors here cited will be found in the monographs of Thayer and Hewetson, of Ziemann, and of Mannaberg, already quoted.

dently representing the nucleus of the parasite. On the inner border of the ectoplasm of younger parasites they found a deeply staining body which they regarded as the early form of the nucleus. Their plates accurately depict the growth of the "endoplasm and nucleus" up to the presegmenting stage, into which they were unable to follow it. This demonstration of the nuclear body was, in the minds of competent observers, the beginning of the end in the controversy regarding the truly parasitic nature of the malarial organism.

Using the same technical methods, Grassi and Feletti, in 1890, described in the larger parasites "a large vesicular nucleus such as is seen in many rhizopods." This nucleus was usually eccentric, possessed a very thin, often indistinct membrane, and an intranuclear network filled with a semi-fluid substance. The intranuclear network exhibited a nodal thickening resembling a nucleolus, which was sometimes round or often showed several radiating filaments stretching toward the nuclear membrane. This nucleus was not found in young parasites. The authors believed they could discover evidences of direct division of the nucleus, beginning 12 to 16 hours before segmentation of the body. The nucleus of Grassi and Feletti undoubtedly corresponds to the endoplasm of Celli and Guarnieri.

In 1891 Romanowsky published his observations on the structure of the tertian parasite as demonstrated by his special staining method. He described the nucleus as a colorless central area in the parasite, in which appeared a smaller body staining of a carmine violet color, the "nucleolus" or chromatin of the nucleus. In the larger parasites he described the development of fibrillar chromatin bodies in the nucleus, indicating a process of indirect division. These filaments were indistinct but gave the mass of chromatin a less compact appearance. The diaster stage is roughly indicated in one of the sketches. Romanowsky described, also, "quinine forms" in which the clear zone of the nucleus was wanting, this structure fading insensibly into the body of the parasite, while the chromatin was subdivided into many fine granules. To judge from the drawings, these "quinine forms" appear to be identical with Gautier's presegmenting forms (see p. 440). Romanowsky mentions no stage of the parasite which failed to show a nuclear body stainable by his method.

Sacharoff, in 1891, observed the "nucleolus" lying in a clear nucleus in specimens of æstivo-autumnal parasites stained by gentian violet, and noted the disappearance of the nucleus just before segmentation. In 1893 he applied Romanowsky's method to the minute study of the nucleus and described a fibrillar structure which occasionally showed karyo-

kinetic figures. He found, further, that the flagella of the parasite stain like the chromatin of the nucleus, and concluded that the flagella are separate chromosomes of the karyokinetic nucleus, extruded from the parasite under the influence of cold. In 1895, he reported a further study of the nucleus, and described the "extrusion of chromosomes" (exflagellation) from the malarial parasites of young crows. The parasites of these animals were found specially adapted to the purpose, as their nuclei are large, chromatin filaments are distinct, and flagellate bodies are found in the blood immediately after shedding. In these parasites he depicts intracellular formation of flagella and the extrusion of all the chromatin from the body of the parasite in the form of flagella. The author refers (1895) to the studies of Sala on the eggs of *Ascaris megalocephala*, in which indications were found of an active movement on the part of the chromosomes, and to the conclusion of Strasburger that the changing position of chromosomes in some vegetable cells results from an active movement on the part of these structures. It may be added that in recent years an extrusion of all chromatin in the form of flagella has been observed in various forms of coccidia by several investigators.

Bastianelli and Bignami described the minute structure of the æstivo-autumnal parasite in specimens stained by hæmatoxylin. In the young parasite they describe as "endoplasm" the large central achromatic area through which shines the hæmoglobin of the infected cell, while the deeply staining peripheral granule was said to consist of chromatin. This nuclear body, or endoplasm, possesses no membrane and exhibits no special structure. The chromatic granule increases in size as the parasite develops and the clear endoplasm acquires a light bluish tinge, partly obscuring the hæmoglobin. Later, when the pigment has gathered in a single mass, the body of the parasite becomes homogeneous and the chromatic granule disappears. These changes mark the beginning of the reproductive phase, and may be followed very shortly by segmentation. During segmentation the chromatin reappears scattered through the body in fine particles, about each of which a ring of chromatophilic substance gathers. A small remnant of the endoplasm is left unutilized in the segmenting process. The spores at first contain no endoplasm, which appears only in the young parasite. The authors do not find, either in their own preparations or in the drawings of others, any definite structures recalling a true nucleus. The disappearance of the chromatin before sporulation they find to be analogous to a similar phenomenon in the Gregarinidæ and Coccidia, while in the Oscillariæ one or more disseminated granules of chromatin represent

nuclear bodies similar to those seen in the malarial parasite. The nucleus of the malarial amoeba, they believe, never assumes the vesicular or resting stage on account of the rapid succession of generations. In the crescents, with rare exceptions, they found no chromatin, and therefore regarded these bodies as sterile.

Mannaberg (1893, 1899) followed the development of the nucleus, as described by Celli and Guarnieri, in specimens stained by hæmatoxylin, and by a special procedure of his own. He was unable to find evidence of a karyokinetic division of the nucleus.

Ziemann studied the structure of the tertian, quartan, and æstivo-autumnal parasites by means of his modification of Romanowsky's stain. He followed minutely the changes in the nucleus in each variety, and described many minor variations which may be of value in differential diagnosis. At a rather early period of the cycle the solid chromatin granule was usually found to become less compact and was sometimes divided into two or three portions. With the disappearance of amœboid motion in the parasite the chromatin is usually divided into many fine filaments or spindles from which are derived, during the full-grown and presegmenting stages, an increasing number of secondary chromatin bodies of variable position and contour, but eventually forming the nuclei of the young spores. He found the eccentric position of the nucleus in the young tertian and its central position in the quartan parasite to be very constant differential characters in these organisms. In the "full-grown" stage of the parasite he found the chromatin more difficult to stain. The parasites which failed to exhibit a mass of chromatin he regarded as sterile. He first described appearances which recalled the chromatin filaments and mitotic figures of Romanowsky, but later concluded that no distinct traces of a true karyokinetic process could be demonstrated in these parasites. After a growth of 16 to 24 hours, the chromatin mass was found to break up into a number of spindle-shaped granules, which showed a very inconstant arrangement. Meantime the limits of the nucleus became very indistinct. In many parasites the nucleus and chromatin disappeared, the parasite increased markedly in size and presented a rich deposit of pigment grains in active vibratory motion. These forms he regarded as sterile, and included among them the elliptical and large oval bodies, in most of which he was unable to demonstrate any traces of chromatin. In a few crescents obtained from the bone-marrow, 11 hours after death, he was able to demonstrate a more or less compact mass of chromatin, but always of reduced bulk.

Okintschitz has described the nucleus of the young forms and the fine

structure of the malarial parasite in specimens stained by eosin, methylene blue, and safranine. In the young parasite the nucleus was found to be a compact mass which, in the tertian parasite, failed to stain with methylene blue, but in the æstivo-autumnal variety stained densely with this dye. The further changes in the nucleus were not fully traced.

Marchoux describes the nucleus of the æstivo-autumnal parasite of Senegal in specimens stained by eosin and methylene blue and by a mixture of thionin and carbolic acid (see p. 431). In the early ring-shaped organism the enclosed substance was regarded by the author as the nucleus, the deeply staining eccentric body as the nucleolus. Sometimes two nucleoli were found at opposite poles of the parasite, an appearance which he was inclined to refer to a process of conjugation in view of the fact that later phases exhibited a single nucleolus. In the full-grown stage the nucleolus assumed a position in the centre of the nucleus, gradually dividing into a number of smaller bodies arranged in the form of a wreath. The later changes were not followed, as the parasites disappeared from the peripheral blood.

Gautier, in 1895, reported a study of the malarial parasite of the Caucasus in specimens stained by Romanowsky's method. He finds that the nucleus consists of a vesicular portion and a violet-staining mass of chromatin. The chromatin body is usually surrounded by a narrow, "milky zone," which is sometimes continued about the entire vesicular nucleus. In the ring stage the hæmoglobin shines through the vesicular portion of the nucleus. In many parasites at various stages the "milky zone" is invisible. With the beginning enlargement of the body of the parasite the chromatin changes from a small compact body to a less compact oval mass of granules. It sometimes early breaks up into two or three portions, or it may consist of a single mass of small granules. In some of Gautier's drawings these granules are placed in the centre of the hæmoglobin mass which he regards as shining through the vesicular nucleus. In bodies probably representing the early presegmenting stage of the parasite he describes the development of a reticular structure of the parasite and the total disappearance of chromatin particles. Later the chromatin grains reappear in the meshes of the reticulum. In crescents and ovoids he found numerous small chromatin granules. In the crescents these granules appeared to be much more minute than in the ovoids. Many large parasites without chromatin he regarded as dead.

The nuclear changes which I have observed in specimens stained by Nocht's method have been detailed under the descriptions of

species. They were largely in accord with the observations of Gautier and of Ziemann.

I find that the nucleus of the parasite belongs to the "distributed type" of protozoan nuclei (p. 436), consisting of granules of chromatin and, certainly in the older and possibly in all stages, of an achromatic substance in which the granules are embedded. While the claim of Bastianelli and Bignami must be admitted, that the parasite possesses "no true nucleus," in the metazoan sense, it exhibits nevertheless all the nuclear structures required in some protozoa.

Neither the nucleus nor the achromatic substance appears to be necessarily connected with the interior of the ring, which is the form assumed by the young and vegetative parasite. It seems most probable that this form represents a true ring, or if not, the ring is bridged by a substance which has no essential nuclear relations.

I find no forms in the fertile cycle of the parasite in which chromatin cannot be demonstrated. Various intra-cellular and extra-cellular forms devoid of chromatin are for that reason necessarily regarded as sterile.

Although there appears to be abundant analogy in the nuclear changes in the parasites of birds and in some closely related coccidia, to indicate that the human parasite may divide by a modified form of karyokinesis, I could find no sufficient ground for applying this term to the series of nuclear changes observed in the presegmenting parasites in man.

Labbé, Danilewsky, Sacharoff and others, in the blood parasites of animals, and Simond and Siedlecki, in various coccidia, find that the chromatin regularly appears at some stages in the form of fibrils, and that these may describe figures rather closely resembling the mitoses of metazoan cells. Romanowsky claimed to have seen distinct chromatin filaments, and sketches imperfect mitotic figures in the tertian parasite. Ziemann relinquishes a similar claim in his second article, admitting that no distinct mitotic figures are to be demonstrated in the human parasite. Gautier's sketches show nothing of these filaments. In my specimens from fresh malarial blood chromatin was never seen in the form of a filament, all elongated masses being invariably of granular structure. On the other hand, when exflagel-

lation occurs with the human parasite, the chromatin becomes filamentous, figures resembling monasters are produced, and the chromosomes are extruded as active flagella. This process is entirely in accord with the changes depicted by Sacharoff in the parasites of birds.

It appears, therefore, that in the fertile cycle of the malarial parasite division occurs by a very simple process which may be likened to amitosis, the only visible changes in the chromatin being subdivision and fusion. In another cycle of development, adapted for the extra-corporeal growth of the parasite, division occurs by a modified form of karyokinesis, the chromosomes leaving the parent cell to fertilize other individuals. Some of the structures seen in coccidia by Simond and Siedlecki have been interpreted as showing the fertilization of one parasite by the flagellum of another. MacCallum,¹⁷ working with the blood of infected crows, repeatedly saw free flagella enter other parasites in which they became lost, and he was able to repeat this observation in a case of human æstivo-autumnal infection. Evidence is therefore gradually being gathered to determine the true significance of flagellation and to locate in the proper place the function of karyokinesis in the malarial parasite.

V. THE CRESCENTIC BODIES.

While the results of recent studies of the coccidia (Simond, Siedlecki, Schaudinn) bear on some obscure points in the biology of the malarial parasite, the full significance of the crescentic bodies, even in the coccidia, has not yet been demonstrated, although the position of these bodies in the developmental cycle has been determined. In various coccidia it has been shown that there are two cycles of development, one, the sporulating cycle, leading to the development of encysted bodies, the other, asporulate and parthenogenetic, leading to the development of crescentic and flagellate bodies. The individuals of the sporulating series are capable of reproduction in the host, but in the asporulate series, the crescents and flagellate forms are very fragile, disappearing rapidly when exposed to unfavorable conditions, and alone are incapable of self-perpetuation. There is evidence in the coccidia that some of the crescentic bodies represent the female element and require fecundation by the flagellum or male element, in order to become fertile.

¹⁷ *Journal of Experimental Medicine*, 1898, iii, p. 117.

MacCallum's observations on *Halteridium*, a crescentic parasite of birds, indicate that these crescentic bodies are of two varieties, one, the male, producing flagella, the other, the female, uniting with a free flagellum and developing into a motile form called the "vermiculus."

Further evidence on this point has been furnished by Ross, who found that when the blood of birds infected with *Proteosoma*, a species of parasite closely resembling the malarial amœba, reaches the stomach of the mosquito, many of the organisms become flagellated. A few days later he finds in the stomach-wall of the mosquito certain large encysted pigmented bodies containing many rod-like structures and some "black spores," which on the rupture of the cyst gain the general circulation. In the salivary glands of the insect these "germinal rods" may be found in large numbers. Ross was able to infect young birds by subjecting them to the bites of mosquitoes fed on blood containing *Proteosoma*, but was unsuccessful with *Halteridium*.

Grassi, Bignami, and Bastianelli, have confirmed and extended Ross's observations. These investigators succeeded in conveying the æstivo-autumnal infection from one human being to another by means of a particular variety of mosquito, *Anopheles claviger*, the "dapple-winged" mosquito described by Ross. Moreover, they fed their mosquitoes on blood containing crescents, showing that these bodies are capable of further development in a new host. In the mosquito they observed, as Ross had done, exflagellation of the crescents, development of encysted bodies in the stomach-wall, discharge of "germinal rods," and their accumulation in the salivary glands of the infecting insect. Later they succeeded in transferring the tertian parasite in the same way, the large hyaline forms furnishing the flagella in the mosquito's stomach. They found no bodies resembling the vermiculus of birds, and it has not been shown how the parasite pierces the wall of the stomach. It is thus clear that the crescentic body is a form of the parasite adapted to further development in a new host.

Of the mode of origin of the crescents in man there is still nothing definitely known. In support of Mannaberg's theory that they are conjugation forms resulting from the union of two ring forms, no new facts have been observed. Grassi and Feletti, who, in 1891, claimed that in birds a certain number of crescents were undoubtedly produced by conjugation of younger forms, have apparently not insisted upon the correctness of this view. On the contrary, the studies of the development of coccidia in the rabbit, salamander, cuttle-fish, and other animals, strongly oppose Mannaberg's theory, for in these organisms, which, according to Metchnikoff, are clearly related to the malarial parasite, the

crescentic form is produced in an entirely different manner, by the segmentation of a large spheroidal body into several small but fully formed crescentic bodies. No similar parent bodies have been described in the blood or tissues of the human subject.

Celli and Guarnieri, in 1889, and Canalis in 1890, depicted the young forms of the crescentic bodies as small, narrow crescents with considerable fine pigment, lying within slightly altered red cells, and traced the development through a gradual increase in size, with destruction of the hæmoglobin of the infected cell, up to the adult crescent. From the adult crescents, ovoid, elliptical, and spheroidal bodies may then form, and these frequently become flagellated. These phases of development have been very generally accepted, and are largely in accordance with analogous processes in various coccidia. There is, however, a lack of agreement regarding the relation of the ovoid and spheroidal bodies to the crescentic forms. When examined in the fresh condition crescents are frequently seen to assume the spheroidal form, and if a little moisture is added the spheres may extrude flagella. Occasionally, however, the spheres or ovoids may be seen to revert to the crescentic form, as described by Ziemann and others. Now, crescents of almost any size may be made to assume the spheroidal form, from which it appears that this body is not always to be included in the natural developmental series of the crescent. I have seen spheroidal bodies develop from crescents about which there was hardly a trace of hæmoglobin, while in other cases the spheroidal body did not occupy more than two-thirds of the red cell. The young crescents which appear in the blood on the fourth or fifth day of the paroxysm have, in my cases, been of small size, rather broad, and often no longer than the red cell. They are often distinctly oval or spheroidal in the stained specimen. During the fifth to the seventh days they gradually increase in size, with progressive destruction of hæmoglobin, and finally assume the elongated crescentic form, without hæmoglobin. My conclusion, therefore, is that the ovoid and spheroidal bodies seen in the ordinary stained specimen are usually younger forms than the elongated crescent, and that the spheroidal bodies which form in shed blood may be derived from crescents of almost any age. The quantity of hæmoglobin about the spheroidal

body would seem to be a reliable indication of the age and original form.

In 1889 Canalis described a form of *segmentation* in crescents. The segmenting bodies were elliptical in form and discharged eight or ten rather large spores. At the same time, Celli, and Marchiafava and Golgi, were inclined to believe that crescents might sporulate in the blood, but were not certain that they had ever seen such forms. Antolisei and Angelini, in 1890, confirmed Canalis's observations, stating, however, that the new spores possess a double contour. Grassi and Feletti, and Sacharoff, believing that the crescents represent a separate species of organism (*Laverania malarie*), accept of necessity the hypothesis of their sporulation, but have not positively identified segmenting forms in the blood. Lewkowiez (1897) reports that he has seen two crescents in the act of sporulation in the blood, and in the splenic blood he describes some segmenting crescents containing as many as thirty spores. The transverse subdivision of crescents has been observed by Grassi and Feletti, Mannaberg, Ziemann, and others. Ziemann, however, regards this process as unquestionably not of a reproductive nature. The transverse segmentation of crescentic bodies has been clearly demonstrated in coccidia (Jackson Clarke), but the fate of the segments is not shown, and while there are indications that the crescentic bodies of coccidia may be multiplied to some extent in this way, the same evidence clearly shows that the process is exceptional.

The various phases of gemmation and budding described by many writers have never been strongly urged as a natural method of reproduction of crescents.

Accordingly, opposed to a moderate number of inconclusive and often uncertain observations favoring the segmentation of crescents, there are entirely negative results from the vast majority of observers.

Various studies of related protozoa indicate that crescentic bodies, after fertilization, regularly proceed to further development with encystment and the production of an entirely different form of the parasite, but sometimes leading to autoinfection of the same host. Although there is no satisfactory evidence that the malarial crescents can develop further in the human being, it is by no means certain that their formation and development are entirely innocuous to the patient.

Not a few observers have connected certain febrile paroxysms with the growth of crescents. Golgi in 1889 referred some forms of fever at long intervals to the development of new broods of crescents. Laveran still (1899) holds that crescents alone, without the presence of other forms, can be associated with a febrile paroxysm. Canalis, who claimed to have found sporulating crescents, connected the segmentation of these bodies with paroxysms recurring in three or four days. Celli and Sanfelice, and Grassi and Feletti not infrequently observed paroxysms in birds associated with the appearance of crescents only in the blood. Lewkowiez believes that the development of crescents may produce quotidian or tertian fever, or paroxysms at long intervals. He believes, also, that crescents are not so refractory to quinine as is generally supposed; that they disappear under quinine after a variable period, and that the long-persisting forms are new individuals reproduced in the viscera from day to day.

Most authorities, however, prefer to attribute the irregular paroxysms to the production of a limited number of ordinary amœboid parasites, which sometimes fail to reach the general circulation in demonstrable numbers.

The possibility that the development of crescents is associated with a febrile paroxysm is by no means disposed of by the proof that crescents do not sporulate. There is almost certainly a secondary cycle of development of the parasite leading to the formation of crescents, and this cycle may well be several times repeated, each time with fever. For each crescent destroys a red cell, and the crescents in the blood are sometimes as abundant as the brood of young amœbæ. Although most cases of fever at long intervals are probably simple relapses, it is impossible to deprecate wholly the tendency to regard some of these paroxysms as evidence of a second cycle of development in the parasite leading to the formation of crescents. In the Montauk series I was frequently surprised to find only young crescents in the blood associated with mild seizures at irregular intervals. It was especially noted in some cases in which crescents persisted in the blood after fever had subsided and while quinine was still being administered, that shortly after a mild chill numerous young crescents appeared in the blood.

Some *morphological features* of the crescentic bodies are still of

active interest. The early observers, who believed that crescents were an encysted form of the parasite, frequently depicted these bodies with a distinct double contour, representing a sharply defined membrane. Without entering into the details of opposing views, it may be said that this extreme claim of a distinct double membrane has been slowly abandoned, and the most that is maintained is the existence of a condensed outer border about the crescent. I find that this outer border may be colored red by strong staining with eosin. That the reddish border thus developed is not identical with the membrane of the red cell appears from the fact that it invests the concave side of the crescent where it may be widely separated from the projecting bow of the red cell. The remnant of the red cell which stretches like a bow across the concavity of the crescent, while usually single, appeared double in one of the specimens. The ends of the bows then overlapped, each enclosing a little more than one-half of the crescent (Plate XXX, Fig. 23).

The identity of the bow with the membrane of the red cell has been accepted without question, and its development in young specimens leaves little doubt of this origin, but why it should increase in dimensions with the growth of the crescent and why it is occasionally double are at present obscure questions.

The application of Nocht's method to the crescentic bodies furnished valuable additions to the knowledge of these forms. Ziemann found the vast majority of crescents to be entirely free from chromatin. Gautier, however, working with Romanowsky's procedure, was apparently the first to demonstrate the presence of chromatin in any large proportion of crescents in human blood. In the young ovoid or elliptical forms he found a well-marked group of rather large granules of chromatin. In the full-grown crescent a single group of very fine granules lying in the centre of the body and often partly obscured by the pigment mass, could, in the majority of specimens, be fully identified.

With Nocht's method I have been able to demonstrate chromatin granules in the vast majority of crescents in all stages (Plate XXX, Figs. 17-23). In the younger forms the granules were usually larger and more distinct than in the older forms. In the adult crescents the chromatin was usually found in a single rather compact mass of

minute granules, which was usually much obscured by overlying pigment. Occasionally the chromatin mass lay to one side of the wreath of pigment, in which case it was very easily identified. In a few specimens in which the pigment was diffusely scattered over the crescent, the chromatin was very clearly visible (Plate XXX, Fig. 22).

Two groups of chromatin granules were seen in a moderate number of crescents, in some of which the pigment was arranged in the form of the figure 8. In these specimens both groups of chromatin granules were inclosed in a single mass of clear achromatic substance (Plate XXX, Figs. 21 and 23).

In a few specimens it was impossible to detect any traces of chromatin, indicating that these particular forms were sterile. Such forms, however, were not more numerous than were chromatin-free parasites in ordinary cases of tertian or æstivo-autumnal infection, while they were difficult to find in specimens in which the staining had been especially successful.

Nocht's stain very clearly demonstrates an elliptical relatively achromatic area in the centre of the crescent in which the pigment and chromatin are usually included. The line of demarcation between the bluish staining poles and the achromatic area was often very sharp after the application of this method. Occasionally the achromatic area was found well out in one pole (Plate XXX, Fig. 20).

The application of Nocht's method to other forms of the parasite greatly increases the number of forms in which evidences of approaching segmentation may be found. In my specimens of crescents no variation in the chromatin granules or mass was detected pointing to a reproductive process. The older and larger the crescent the smaller and less distinct these granules became. In some spheroidal bodies of a fatal case, however, the body of the parasite was distinctly reticulated, although the chromatin grains remained in a single mass.

VI. EXTRA-CELLULAR PARASITES.

That the young parasite during its passage from the parent rosette to the new red cell is sometimes caught in the plasma in both fresh and dry specimens is evident from the reports of various observers.

The possibility of identifying such young free forms in the fresh

condition may, however, be doubted. Ziemann, commenting on this point, says that "only in the beginning of his studies of malaria did he venture to identify young amœboid organisms in the plasma of fresh blood." Celli and Guarnieri (1889) have sketched the appearance of young extra-cellular bodies, including forms of at least two species of parasites, in fresh blood stained by methylene blue after their special procedure, but many of these, especially the pigmented ones, were undoubtedly separated from the cell during the manipulation. In preparations of fresh blood, parasites so frequently pass from the cell into the plasma that it may be doubted if any accurate estimate of the number of extra-cellular forms in the circulating blood can be obtained by this method of examination. In some cases in which I have seen suspicious extra-cellular bodies in fresh specimens, Nocht's stain failed to show any extra-cellular parasites whatever. If reliance be placed upon dry specimens stained by this method, and the demonstration of a distinct nucleus be required, extra-cellular parasites must be looked upon as a comparative rarity, but may undoubtedly be seen in exceptional cases. Gautier and Ziemann depict such forms, while mentioning their rarity. Romanowsky could find young extra-cellular tertian parasites only in patients taking quinine. The same rule appears to hold with the later stages of the parasite, extra-cellular parasites being found with extreme rarity. In fresh specimens a considerable number of large forms appear to be extra-cellular, but these, in stained specimens, usually show some enclosing remnant of a red cell.

Various *sterile forms* of parasites described at length by Golgi, Ziemann, Bignami and Bastianelli, and others, while usually endoglobular, are sometimes seen in the plasma, and in dry specimens may be found to be distinctly extra-cellular. The characters of these sterile forms are, according to Ziemann: (1) increase in size, (2) loss of amœboid motion, (3) greater abundance of pigment and increased vibratory movement of pigment granules, (4) markedly hyaline appearance in stained specimens, (5) complete or nearly complete absence of chromatin. Ziemann, however, includes among the sterile forms crescents and spheres derived from crescents, which do not properly belong in this class, as they contain chromatin and under

suitable conditions are capable of further development. Other sterile forms described by Ziemann were found most abundantly in the splenic pulp after death. These were spheroidal bodies, of large size, hyaline aspect, vibratory pigment, and deficient supply of chromatin.

Similar large forms have often been described as derived from full-grown quartan and tertian parasites, and their extrusion from the red cell has been followed in specimens of fresh blood. The extra-cellular position of most of these forms appears therefore to be artificial. Working principally with dry specimens, I have always had great difficulty in finding any of these large extra-cellular forms, and believe that they are extremely rare in the circulating blood. In specimens of fresh blood, however, which have been allowed to stand for 1 to 24 hours such forms become rather numerous.

Vacuolation has also been frequently described in these large sterile parasites. In the earlier observations of parasites in the fresh condition the nuclei were sometimes mistaken for vacuoles, an error against which Golgi warns. In stained specimens I have very rarely been able to identify vacuolated parasites and believe that their identification in fresh blood is usually very hazardous.

The relation of the parasite to the red cell still remains a matter of dispute. Laveran holds that the majority of parasites are merely attached to the surface of the cell, though some are found within its substance. The crescentic bodies he regards as strictly intra-cellular.

The frequent appearance of the projection of the æstivo-autumnal ring beyond the circumference of the red cell has led many to believe that this parasite, at least in its early stages, is merely attached to the cell. Marchiafava and Bignami, however, point out that the æstivo-autumnal ring in the fresh condition never sends pseudopodia beyond the edge of the cell, and may be seen dipping down or swimming at different levels in the cell.

Gautier very accurately depicts the appearance of the ring projecting beyond the cell, and there seems to be no good reason to doubt that such parasites are merely attached to the cell. It by no means follows, however, that later stages of the æstivo-autumnal ring are not found within the cell, as described by Marchiafava and Bignami. It is generally accepted that the tertian parasite lies within the red cell,

yet in many tertian cases the body and especially the nucleus of the parasite appear to project beyond the border of the cell, even more distinctly than in the case of the æstivo-autumnal ring. Such attached forms seem to be more frequent in the actively amœboid stage, and in cases taking quinine. In fresh specimens which have been allowed to stand and are afterwards dried and stained, the parasite may be found in various stages of extrusion, and similar appearances of projection of body and especially of nucleus beyond the cell are numerous. Mannaberg probably correctly expresses the facts in this matter as follows: "The young parasites swim in the plasma for a very short time and soon become attached to red cells. They remain attached to the cell for a time but soon penetrate within, where their further development is completed."

It is probable that the æstivo-autumnal parasite remains attached to the cell longer than the tertian, possibly because it is less actively amœboid.

VII. ON A FORM OF CONJUGATION OF THE TERTIAN MALARIAL PARASITE.

In four cases of tertian infection I have encountered appearances in the blood which seem to admit of no other explanation than that of conjugation of malarial parasites. In a considerable number of other cases similar appearances were found, but much less frequently.

The blood in these cases showed a moderate number of young rings and a large number of half-grown and full-grown forms. A great many red cells showed double infection with young rings. In many instances these rings were entirely separate, each exhibiting a single large granule of chromatin. Many cells, however, contained two rings, which were clearly *fused together along one segment of the ring*, and two large chromatin granules were then invariably found at different points in the rings (Plate XXXII, Figs. 2-5). The fused parasites usually differed in appearance. One was a large delicate ring with a thin bow, and chromatin granule of moderate size, while the other was a coarser body with thickened bow, enclosing little or no hæmoglobin, and exhibiting a large chromatin granule (Plate XXXII, Figs. 3-5). These differences between the two conjugating parasites could not always be found. Among the single rings, the

two forms of young parasites were often distinguished, but no single rings could be found containing two *equally* large chromatin granules, while every red cell that exhibited two large and equal chromatin granules contained also two distinct rings. It appeared therefore that the bodies of many parasites had become fused together, while their nuclei remained separate. Occasionally the two chromatin granules were found close together, but no distinct signs of a fusion of chromatin were found at this stage.

On examining the parasites in later stages of development, most of them were found to have lost the ring form, and to have spread out into a large number of threads, with nodal thickenings, variously curled in the red cell. These threads evidently represented the pseudopodia of a very active amœboid stage. The chromatin masses were now subdivided into 10 to 12 granules, but in the majority of the cases these masses were far apart and showed no tendency to unite. In many cells, however, the amœboid figures were less marked, *and the masses of chromatin lay side by side united by a little achromatic substance*. Later some parasites were found in which the two groups of rather large chromatin granules lay in *immediate apposition, surrounded by achromatic substance*. This phase was marked by a distinct reduction in the length of amœboid figures (Plate XXXII, Figs. 6-11).

Many older, spheroidal, hyaline forms, belonging to this same brood, were found in these cases. All the older hyaline forms were single and exhibited a single large group of fine chromatin granules. Not one cell harboring two full-grown parasites could be found in prolonged and repeated search through several slides.

The question therefore arises, what became of the very large number of twin parasites seen in all the younger stages? In one of the Montauk cases the two broods were of different ages, one approaching segmentation *and all single*, the other less than half-grown and *almost invariably twinned*. Can twinning occur in part of a brood and not in its oldest members, or in one brood extensively and not at all in its predecessor? While such physiological variations are possible, they appear extremely improbable, and one is forced to the conclusion, merely from the absence of older twinned parasites, that conjugation

occurred. Whatever interpretation may be placed upon this peculiar absence of older twinned forms, the finding of all stages of union, first of the bodies, later of the nuclei, as illustrated in Plate XXXII, appears to admit of no other explanation than that of conjugation.

The further examination of these and other specimens developed some other peculiarities of interest. Single parasites of each of the above types could apparently be traced through later stages of development. The small, coarse, densely staining body remained comparatively compact throughout its development. It was rarely found in distinct ring form, enclosing hæmoglobin, but often exhibited coarse amœboid processes. It was usually of smaller size than the average tertian parasite, but the infected red cells were swollen and pale. In the full-grown stage this body was compact and densely staining, with rather distinct chromatin granules, but I could not trace it up to a sporulating body (Plate XXXII, Fig. 13). In many respects these large forms resemble the quartan parasite, but the infected red cell is swollen, the pigmentation is not marked and the majority of them, in younger stages, have been found to conjugate with the ordinary tertian rings.

The other type of parasite of the conjugating pair also frequently developed singly, but I am not certain that it reached sporulation. The young forms showed the delicate ring shape with thin bow (Plate XXXII, Figs. 3-5). The larger rings enclosed much hæmoglobin and often exhibited amœboid figures. The infected cells were distinctly swollen and pale. The full-grown form stained very slightly and appeared hyaline, while its chromatin was slight in quantity and minutely subdivided. No presegmenting bodies could be found in these cases which appeared to show the characters of this pale full-grown parasite. All presegmenting bodies and rosettes were either densely staining (before distinct reticulation), or of large size and with abundance of chromatin, the former developing from the compact forms described, the latter apparently from the conjugating parasites but possibly also from the single rings. Possibly the pale hyaline full-grown forms with finely subdivided chromatin were destined to become flagellate forms (compare MacCallum's two varieties

of crescents). Some of the above features are illustrated in the accompanying drawings (Plate XXXII).

Some considerations which do not favor the belief in a process of conjugation require mention:

1. The suggestion naturally arises that the presence of two masses of chromatin does not necessarily mean the presence of two parasites in one red cell.

From a long series of observations on the character of the chromatin in young tertian parasites I must admit that this objection is partly valid. The young tertian parasite, in some cases, may be found to contain two masses of chromatin. In the young compact body (mentioned above) (Plate XXXII, Figs. 1 and 3) these granules when present are large and of nearly equal size, but in the delicate tertian ring I have never seen two distinct and equal chromatin granules. In somewhat rare instances the ring shows an accessory granule of small size in the neighborhood of the main granule, but never, in my observation, have two large granules occurred in a single thin ring-shaped tertian parasite. The significance of these double granules is not clear. The appearance of two, very small, compact, spore-like bodies partly fused together, as may occasionally be seen, indicates that such forms may sometimes result from the early union of the bodies of two very young parasites. The accessory granules in thin ring forms have always appeared too small to have been derived in the same way. It seems probable that such accessory granules may result from the incomplete fusion of the original granules which go to form the chromatin of the spore, or, in other instances, from a precocious subdivision of chromatin in the young parasite, as suggested by Ziemann.

The presence of two nuclei in some very young compact parasites before they enter upon the process of conjugation with the large rings explains the occasional appearance of *three nuclei* about to unite, as seen in some conjugating forms toward the completion of the process (Plate XXXII, Figs. 9 and 10). In one case, the small single compact forms with two nuclei, and large conjugating forms with three nuclei were present in considerable and about equal numbers. That the presence of three, large, entirely separate, subdivided nuclei in

one conjugating form means the union of three original parasites, I do not believe; but the morphological appearances above described indicate that it invariably means the union of at least two parasites.

It appears, therefore, that the presence of two large and equal masses of chromatin in one infected cell indicates, with few exceptions, the presence of two parasites. Rarely three nuclei are seen in conjugating forms, two of which may be derived from two very young compact forms uniting very early, and the third from subsequent conjugation with a thin ring-shaped form.

The further development of young parasites with small accessory chromatin granules may be followed in rare instances. The accessory granule divides as does the main mass of chromatin and later unites with the other to form one clump of granules in the full-grown stage. Throughout these stages the total bulk of these two masses appears not to exceed the average for single parasites, whereas in the conjugating forms the excessive quantity of chromatin in all stages is a very striking feature. In all the examples of such single parasites that I have seen, the unequal size of the chromatin masses was distinct, and there were no appearances suggesting the presence of two parasites in the same cell. These forms, therefore, differ entirely from the conjugating forms above described. I have never seen more than two masses of subdivided granules in a single parasite, whereas three large and equal masses may be observed in conjugating parasites.

A third minute granule may rarely be seen, however, in young rings. Ziemann¹⁸ describes the appearance of multiple chromatin masses in young tertian parasites. He was at first uncertain whether this appearance was referable to the presence of two fused parasites or to an early division of one nucleus, but finally accepted the latter explanation. He describes the separation of one, or rarely two, accessory granules from the original mass in cells infected by single parasites. Sometimes the accessory granule was much smaller than, sometimes nearly as large as, the main granule. All of these appearances I have seen in single parasites, less often in

¹⁸ Ziemann, *Centralbl. f. Bakter.*, 1897, xxi, p. 643.

single members of conjugating pairs, and I agree with Ziemann as to their significance, but the conjugating forms above described are quite different, and do not appear in Ziemann's descriptions.

2. It may be objected, further, that it is impossible to determine when the bodies of two parasites are really united, as one may overlap the other and produce a false appearance of union.

This difficulty is undoubtedly present with some of the young forms, but with others the appearances of the parasites toward the completion of the process, when amœboid motion is subsiding, are, on the contrary, absolutely convincing that the bodies are actually fused. The significance of two large masses of chromatin surrounded by one achromatic zone is also unmistakable (Plate XXXII, Figs. 9 to 11).

3. Again, it may well be pointed out that examples of twin parasites of advanced development, presegmenting bodies and rosettes, are sometimes seen in severe tertian infections, furnishing examples of twinning when conjugation does not occur.

This fact is a matter of common observation, and in my series there are a few cases in which it was especially noted. In one red cell a typical rosette with many spores and a compressed hyaline body without apparent nucleus were observed. In another distended cell were seen one perfect rosette, one imperfect presegmenting body, and one compressed hyaline form.

It may be said of these twins, which proceed to segmentation without conjugating, that they are vastly less numerous than the conjugating forms or young twins seen in the same or other cases. I have, for instance, seen hundreds of conjugating forms within the past few months, but I remember only three or four twinned rosettes seen in as many years.

In the cases showing twinned adult parasites a few younger couples were seen, which showed no attempt to conjugate. The great majority of these young parasites and all the young twins in these cases were the typical tertian *ring-shaped* parasites, while the small compact forms were exceedingly hard to find. My observations on this latter point, however, are not so numerous as is desirable and are still in progress.

4. Finally, the comparative absence of older twinned parasites may be referred to the death and extrusion of one of the twins while the other proceeds to full development alone.

In some gregarines in which multiple infection of cells and conjugation of parasites is common (*Klossia*), one of the parasites often succeeds in dwarfing its companions and alone reaches full development. The dwarfed or dead parasites are then found in the cell alongside the growing form (Wolters, Clarke). In some instances of multiple infection by full-grown or segmenting malarial parasites, I have sometimes seen evidences of compression and death of the younger of two or three organisms. More often both parasites appeared to be equally favored. In any case, the remains of the dwarfed parasite ought frequently to be found if one member of the pair commonly inhibits the growth of the other. In the four cases referred to above, no traces of dwarfed parasites could be found, and while young twins were extremely numerous, all the older parasites were single. It therefore appears impossible to explain the entire absence of older twinned parasites, and especially of traces of any abortive individuals in these cases, on any other ground than that of conjugation.

I find, therefore, that the usual fate of twinning of tertian parasites is conjugation; that twins sometimes grow to maturity without conjugation, for reasons which are not clear, but apparently when both parasites show the usual ring form; that the union sometimes involves three parasites but probably always requires the presence of one or more compact densely staining forms, which do not commonly assume the ring shape, and of one of the typical tertian rings.

A further inquiry relates to the uniformity with which conjugation occurs, and its position as an essential or as an accidental phenomenon in the progress of malarial infection.

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but there is not sufficient evidence on which to base any such claim. The four cases referred to as furnishing numerous clear examples of conjugation were selected on account of the abundance of the conjugating forms, but in many other cases less

numerous though equally distinct examples were seen, indicating that the process is of very frequent occurrence. On the other hand it must be admitted that the majority of specimens from routine cases fail to show any distinct traces of the process; from which it may be concluded that conjugation is probably not an essential feature of the growth of the parasite.

In the four marked cases the infection was unusually rich, one of them showing more numerous parasites than I had ever seen before in benign tertian infections. One patient had just arrived at Montauk from Cuba, in September, two others were suffering from a first attack, in July, in New York City. In one case there was a prompt relapse a few days after quinine was omitted. It is possible that important clinical features may be found to be associated with the presence of conjugating forms, but the observations are too limited to furnish any conclusion on this point.

In æstivo-autumnal infections, in which twinning is very common, I have been unable to trace the parasites through the conjugating period on account of their disappearance from the peripheral blood. Of five cases showing rosettes and presegmenting bodies in the blood, in one were found several twins of these older forms in the same red cell, while in four others no twin parasites were found beyond the ring stage, in which evidences of conjugation, as described by Mannaberg, were occasionally seen. The presence of double nuclei in peculiar æstivo-autumnal rings has been noted. Marchoux suggests that such forms result from conjugation, an explanation which appears reasonable but which is at present without proof. Another more probable explanation has already been mentioned (p. 452).

I have been unable to secure any recent specimens of quartan parasites.

Double rings with fused nuclei are apparently a common form of the young parasite of Texas cattle fever (Theobald Smith).

VIII. ON THE PLURALITY OF SPECIES OF MALARIAL PARASITES.

The belief in a plurality of species of human malarial parasites has been accepted probably by a majority of clinical observers residing in temperate climates, but seems never to have gained uniform sup-

port from those who have studied largely in tropical climates, nor from comparative biologists.

The doctrine of plurality of species is maintained by Mannaberg, Koch, and the great majority of German writers, by Welch, Osler, Councilman, Thayer, Dock, and practically all American writers, and by Golgi, Grassi, Bastianelli, and Bignami, representing the Italian school. A middle ground is held by Kruse, Canalis, Marchiafava, Celli, and Sanfelice, Babes and Georghiu, Danilewsky, and Ziemann (in his early publications), who are inclined to accept the unicist theory, or claim at least that the facts do not warrant the belief in the existence of distinct species; while Laveran, Metchnikoff, Marchoux, Vincent, and some others actively uphold the existence of a single polymorphous species.

Among the pluralists no uniform basis of classification has been established. Those who rely strictly upon the morphology of the human malarial parasites rather uniformly agree upon the existence of three species—quartan, tertian, and æstivo-autumnal. Grassi and Feletti, influenced by the morphology of similar parasites in lower animals, add a fourth distinct species, *Laverania malarix* (yielding the crescentic bodies), as well as *Hæmamoeba immaculata*. Golgi regards the distribution of the parasite in the body of quite as much importance as a ground of classification as its morphology, and therefore makes two groups, one, including the quartan and tertian parasites, which are found principally in the peripheral blood, and a second, the æstivo-autumnal, which is found principally in the internal organs. Mannaberg regards the presence or absence of syzygia, *i. e.*, crescentic bodies, as the chief ground for the separation into species, and recognizes as species which do not produce crescents, (1) the tertian and (2) the quartan parasites, and as those which by conjugation form crescents, (1) the malignant tertian, (2) the pigmented quotidian, and (3) the unpigmented quotidian parasites.

Van der Scheer and Plehn, working extensively in India and Africa, find only two well-distinguished species, (1) the large and (2) the small forms. The former include the quartan and tertian parasites, the latter, the æstivo-autumnal or tropical group of other authors.

It is seen that while there are no distinctly contradictory views among the pluralists, there is an entire lack of agreement in regard to the grounds required for the separation of species. Since there is no room to doubt that a certain stability exists in the three generally recognized species of parasites, practically the question at issue is

whether these species are ever interchangeable, and, if so, to what extent and under what circumstances may one species pass into another. Mannaberg has fully presented the evidence in favor of a plurality of species, without, however, considering many opposing facts, and Laveran has ably supported his own belief in a single species, disregarding much contradictory morphological evidence. While it is unlikely that the question will be fully settled until the extracorporeal form of the parasite has been fully traced, there are some recent observations on the subject which may be profitably reviewed.

The strongest evidence in favor of a plurality of species is found in the results of experiments on the inoculation of malaria, which, when properly controlled, have invariably produced the type of organism found in the specimen of blood used in the inoculation. Mannaberg tabulates 33 experiments of this nature, in 30 of which the inoculation produced the type of organism found in the inoculated blood, while in three the result was doubtful. To these may now be added one case successfully inoculated with the æstivo-autumnal parasite by Zagari and Pace; Sacharoff's infection of himself with the æstivo-autumnal parasite taken from a leech; and two tertian, six æstivo-autumnal, and two mixed infections by tertian and æstivo-autumnal parasites reported by Elting.¹⁹ There are thus at least 42 experiments in which the inoculation of a certain variety of parasite was followed by fever and the growth of the same parasite in the blood. Here must be mentioned also the transference of æstivo-autumnal and tertian infections, successfully accomplished by Bignami, Grassi, and Bastianelli, through the agency of mosquitoes. The invariable reappearance in the infected individual of the type of parasite contained in the injected blood is undeniably strong evidence of permanency of these malarial species. On the other hand, the inoculation experiments by no means prove that the so-called species are immutable *under all conditions*. Accordingly, the passage of the parasite through the bodies of mosquitoes and its reappearance unchanged in subjects thus inoculated by Bignami, Grassi, and Basti-

¹⁹ *Zeitschr. f. klin. Med.*, 1899, xxxvi, p. 491.

nelli, must have far higher value as evidence of the immutability of the species. Yet the same element of doubt attaches even to the latter experiments, which may merely indicate that the proper conditions for the transformation of species have not yet been furnished artificially.

A further line of evidence of the same general character is cited by the pluralists in the immutability of species demonstrated in individuals who have been kept under observation for months. It is a matter of common experience that patients who suffer relapses after long intervals sometimes extending over years, usually show the same type of parasite in the relapse as in the initial attack.

Calandruccio examined a triple quartan infection daily for months and found only quartan parasites, and in two cases crescents were found to persist in the blood for two and six months respectively, without the appearance of any other type of organism. Grassi and Feletti also found no change in the type of parasites in a case of quartan fever examined for two months, and in a case of *æstivo-autumnal* infection examined from October to March.

The permanency of the quartan infections might be expected, but the observations on *æstivo-autumnal* cases are valuable indications that *æstivo-autumnal* infections may, at least in some localities, persist unchanged through the winter. In this field, however, the unicists are able to offer cogent evidence in support of their claims. Antolisei (quoted by Bignami and Bastianelli) has seen patients with *æstivo-autumnal* infection remaining in the hospital all winter manifest tertian paroxysms with tertian parasites in the blood in the spring. They, however, regard these cases as examples of latent tertian infection.

On this same point the observations of Marchoux, during an extensive experience in Senegal, are of interest. He believes that, during the rainy season, in susceptible individuals the cycle of the malarial parasites in Senegal lasts 24 hours at the height of the season, but varies in different cases. In Europeans it tends to shorten and the fever becomes remittent or continuous. In the dry season, the cycle is longer, and the volume of the parasite increases. In many patients with a history of old seizures, who have acquired some immunity, the parasite increases in size and at length, he says, becomes identical with the mild tertian species. During the rainy season when the newly arrived Europeans are suffering from infection with the small ring forms, the native

muleteers, when attacked, all show the large tertian forms in the blood. These observations, while evidently lacking in precision, must be regarded as most significant of the underlying conditions governing the character of malarial infection in the tropics.

Ziemann's²⁰ experience led him to conclusions very similar to those of Marchoux, viz., that the biological and morphological peculiarities of the parasite may be altered by change of climate and differences in individual susceptibility. Thus in a patient who had recently returned from Kamerun, typical quartan fever was found associated with æstivo-autumnal parasites, while in another instance an ordinary tertian fever was developed. Ziemann also noted the prevalence of irregular fevers with small parasites among Europeans, while the natives were suffering from quartan infections. In his monograph on malaria²¹ published in 1898, Ziemann opposes the unicists, and acknowledges himself as a supporter, so far as existing evidence goes, of the doctrine of plurality of species.

My observations at Montauk on cases recently arrived from Cuba, and later in New York City, developed some facts of interest in this connection.

In two cases examined in August large tertian and small æstivo-autumnal ring forms and crescents were found in the blood, but when these cases were examined three weeks later only large tertian parasites were found after a prolonged and repeated search. An irregular administration of quinine had apparently rid the blood of the pernicious type of parasite, leaving the benign tertian form to reappear in the relapse.

Of 335 cases in which parasites were identified in the blood, at Montauk, only 20 per cent (including mixed infections) showed the large tertian organism. But during the past winter (1898-99) I examined the blood of 15 volunteer soldiers who were suffering from relapses of malarial fever contracted in Cuba, and all showed the large tertian parasite only. This same experience has been the rule at various hospitals and dispensaries of this city. In one of these patients the blood was examined in August by a competent observer and found to contain æstivo-autumnal rings and crescents, but in Jan-

²⁰ *Centralbl. f. Bakter.*, 1896. xx, p. 653.

²¹ *Ueber Malaria- und andere Blutparasiten.*

uary only the tertian parasite could be found during a sharp relapse. It is of course possible that mixed infection existed in this case, but there is no clear explanation of the failure to find the tertian parasite in August. Also, to reconcile the fact that the tertian parasite was always found in the above-mentioned relapses with the theory of immutable species requires considerable straining of the facts known in regard to the relative virulence of the species and their relative susceptibility to quinine. It appears more reasonable to suppose that under the influence of cold weather, and gradually increasing systemic resistance, the *æstivo-autumnal* parasite was replaced by the tertian in the relapses suffered by these volunteers. Or it may be suggested that the cold weather alone stamped out the *æstivo-autumnal* infections, leaving only the tertian cases to relapse during the winter.

The disappearance of the *æstivo-autumnal* infections during the late autumn and winter recalls the fact that the distribution of the types of infection is determined by climatic conditions, although, as Laveran puts it, "if the species are separate, there ought to be geographical foci where tertian or *æstivo-autumnal* infections largely predominate, whereas all forms of malaria are commonly contracted wherever malaria is endemic."

The theory of "mixed infections" also has been made to bear a heavy burden in order to support the belief in separate species. In the ordinary type of mixed infection the tertian amœboid forms are associated with crescents, but one rarely finds both amœboid forms, with or without crescents, in the same individual. Yet if the patient is susceptible to malaria why should he retain the mild tertian amœba, while the small malignant forms disappear?

The lack of permanency observed in the mixed infections is also a suspicious feature of the condition. Two types of parasites seldom remain long together in the same subject, one very shortly displacing the other, as shown both by clinical observation and in experimental infections (cf. De Mattei, Calandruccio). As a rule, it is the more highly vegetative tertian parasite which in clinical experience displaces the more malignant *æstivo-autumnal*, but this rule may be reversed in experimental infections. De Mattei saw an old quartan

infection disappear after experimental infection by the æstivo-autumnal parasite, as well as the disappearance of æstivo-autumnal parasites after inoculation with quartan. Gualdi and Antolisei record two cases of quartan infection seen in May, which showed æstivo-autumnal parasites in the autumn.

The frequency of mixed infections is undoubtedly an argument against the plurality of species, showing that there is a very close connection between the sources of the tertian and æstivo-autumnal forms of parasite. In my preliminary report of the Montauk cases there were noted 12 examples of double infection out of 86 tertian cases observed. By a subsequent review of a minority of these specimens the number of mixed infections has been nearly doubled by the discovery of single crescents in tertian cases, or of single large tertian organisms among many crescents. I believe that mixed infections are much more common in the severe cases of the tropics than present reports indicate, and that their recognition depends largely on the time one cares to spend on the examination of the blood.

All the above difficulties may, however, be adjusted to the theory of a plurality of species, and in the absence of more definite knowledge of the extracorporeal form and development of the parasite it is unlikely that the question can be settled on such general considerations as those adduced.

Turning to the comparative morphology of the parasite, the evidence both for and against the plurality of species becomes much more specific. Here the pluralist doctrine finds its chief support and, whatever may be the final outcome of the discussion, it cannot be doubted that the three groups of parasites, quartan, tertian, and æstivo-autumnal, exhibit morphological characters which are to a large extent immutable. Yet the two widely different forms—the æstivo-autumnal rings and the crescents—are regarded as belonging to the same species, and the whole groundwork of a morphological classification is found to be insecure on account of the extreme polymorphism observed throughout the entire group of protozoa. Of this a few details may here be briefly considered.

One of the most striking differences between the tertian and the æstivo-autumnal parasites is the dissimilarity in the staining quality of

their nuclei. Methylene blue stains the nucleus of the young æstivo-autumnal ring densely but fails to stain the nucleus of the tertian ring. This difference probably depends upon an unequal mixture of oxy-chromatin and basi-chromatin in these nuclei. I cannot find that similar differences are recognized in the staining qualities of any two species of coccidia or of gregarines, but somewhat similar differences were noted between different phases of the same species of coccidia by J. Clarke, and by Siedlecki.

The study of flagellated bodies in related protozoa may be found to bear on the question of a plurality of species of malarial parasites. In *Coccidium oviforme* flagella have been found to develop from large spheroidal bodies. This protozoan produces crescents, but these have not been traced through exflagellation (Simond). In *Benedenia ovata* flagella have been found to develop from large spheroidal bodies. This protozoan also produces crescents, but these have not been traced to a flagellating stage. In *Adelea ovata*, on the other hand, the exflagellation of crescents has been observed, but large spheroidal forms producing crescents have not been described (Siedlecki).

It would appear that most coccidia and gregarines produce flagella both from large spheroidal bodies and from crescentic forms. Although the homologues of these forms in the malarial parasites are not fully determined, this fact, if fully established, would strongly indicate that the tertian malarial parasite producing flagella from large spheroidal bodies, and the æstivo-autumnal parasite, with flagellating crescents, are phases of one and the same protozoan.

It may be claimed that if the so-called malarial species are interchangeable, transitional forms ought to be abundantly present in some cases, but these have not been fully demonstrated. Yet it appears by no means certain that in order to establish the unity of the malarial parasite it is necessary to assume the existence of transitional forms. In *Benedenia octopiana*, the formation of crescents is preceded by an entire bisexual cycle of development in which the male element is furnished by large spheroidal flagellate bodies. The intermediate forms of the two cycles in this protozoan differ considerably from each other, there are no transitional forms between them, and yet both belong to the same parasite.

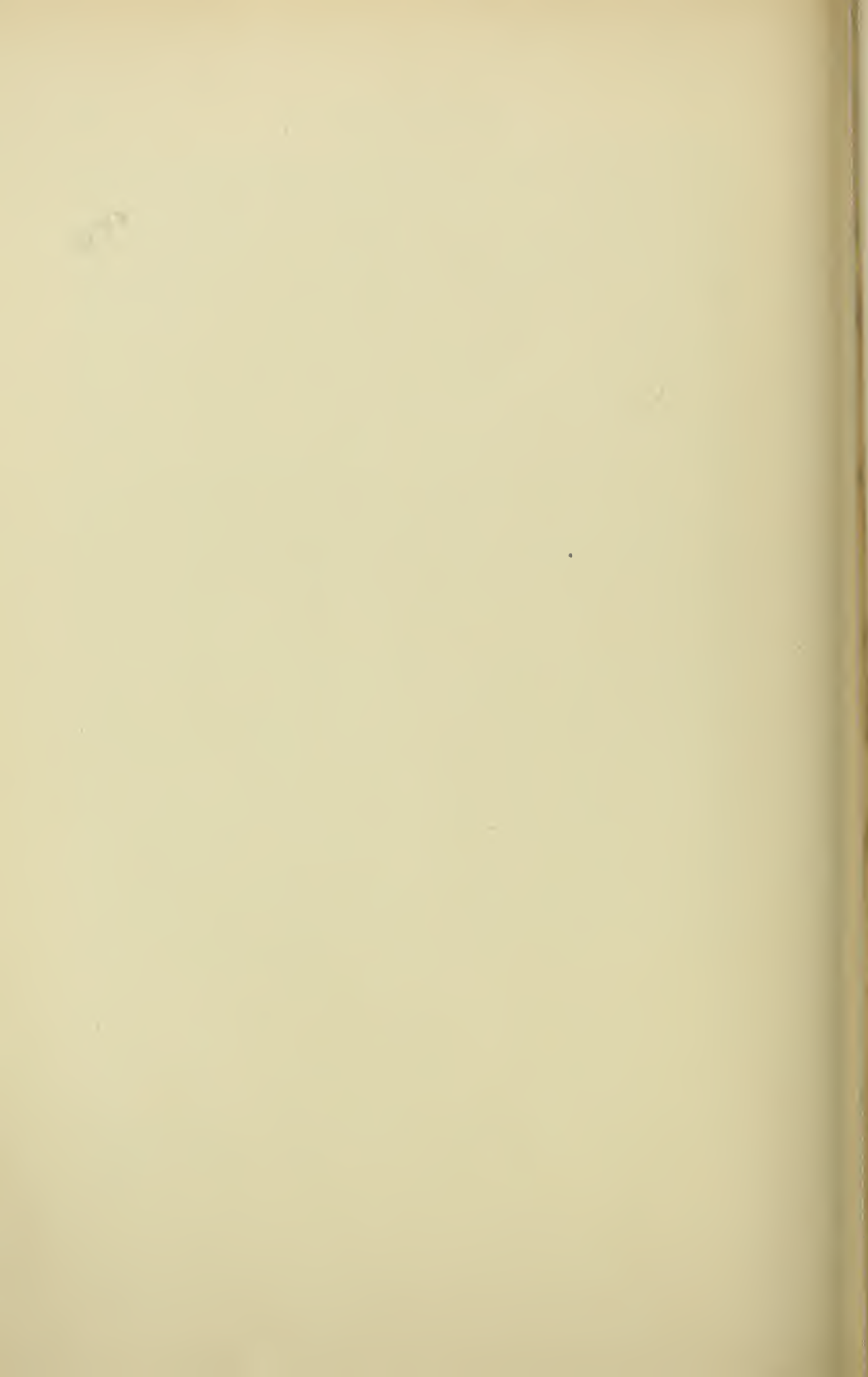
While it is true that no transitional forms between the æstivo-autumnal and the tertian parasite and between the tertian and the quartan parasites have been fully described, there are numerous observations indicating that such forms exist. The slight differences

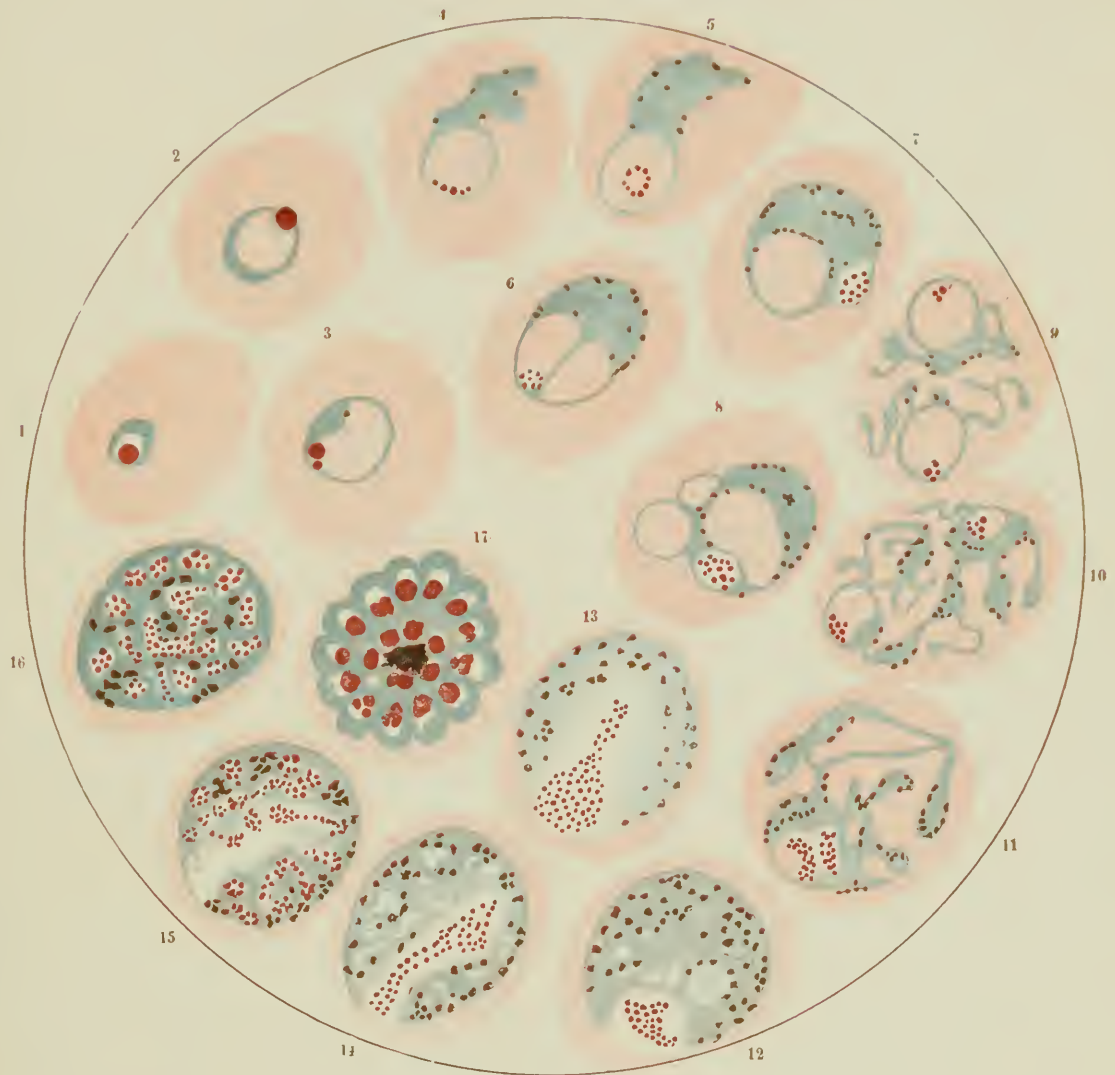
in size, refractive quality and amœboid activity which led Marchiafava and Bignami to separate a quotidian from a tertian æstivo-autumnal species, a position from which they have largely receded, have been noticed and regarded by others as occasional differences in the morphology of one æstivo-autumnal species (Ziemann, Gautier, and others). Marchoux claims to have observed a gradual increase in the size of the æstivo-autumnal parasite during the healthier seasons in Senegal. When one closely examines the parasites seen in the average tertian case of this climate, isolated forms may be found, in greater or less numbers, which closely resemble the quartan parasite. The red cells are not always swollen when infected by tertian organisms, and these parasites are sometimes compact and very richly and coarsely pigmented at an early stage. Of the two somewhat distinct forms of parasites which I find commonly enter into the conjugating pairs of the tertian series, one is compact, of rather small size, and resembles the quartan parasite in some particulars, but the infected cells are swollen.

In examining the blood of volunteer soldiers who were suffering during the past winter from relapses of malarial fever contracted in Cuba, I was early struck with the resemblance which many of the young tertian parasites bore to the young æstivo-autumnal rings. In some of these cases the young tertian rings closely resembled the young signet-ring form of the æstivo-autumnal parasite, exhibiting a very thin bow and a distinct circumscribed swelling of one segment. Their chromatin, moreover, was often found subdivided before the appearance of pigment, although in the vast majority of mild tertian cases seen in New York City, the chromatin of the young tertian parasite is not subdivided till after pigment appears (cf. Gautier). The nuclei of these forms usually failed to stain with methylene blue, but not a few examples were found among the young rings in which the chromatin stained well with methylene blue. The usual swelling of the infected cell was often very slight, or sometimes absent, in these cases. It appeared quite possible to trace the development of these young rings up to the larger amœboid stage when the tertian characteristics become distinct. No crescents were found in any of these cases.

Several explanations may be offered to account for these peculiarities. It may be supposed that the young tertian rings do not necessarily differ from the æstivo-autumnal. This explanation I am unable to accept, finding that the tertian ring, as occurring in New York, invariably differs from the æstivo-autumnal form imported from Cuba, especially in regard to the staining quality of its nucleus (see p. 437). Or it may be supposed that the cases in question were really examples of mixed infection. Yet the suspicious young rings could be traced in development to the large tertian forms, and no crescents were found in any of these cases. Or, finally, it may be supposed that the peculiarities of the young parasites in these cases represented transitional phases between the æstivo-autumnal and tertian parasites. This explanation I am inclined to accept. The observation of 15 cases in which such peculiarities were noted is, however, insufficient to be convincing, and satisfactory grounds for the acceptance of such a belief cannot well be furnished except by demonstrating the complete transformation in the same individual of an æstivo-autumnal infection during the winter through various transitional phases in the morphology of the parasite.

Whichever theory may finally be established regarding the varieties of the human malarial parasite, the evidence would seem to justify the opinion of Kruse, Canalis, Babes, Celli, Danilewsky, and others, who regard the existence of several species as not yet proven, and who find not only in malarial parasitology, but especially from comparative biology, that the phenomena of the disease are more easily reconciled with the existence of a single polymorphous species. Certainly, in many ways, the knowledge of the disease would be furthered by adherence to the unicist theory as a practical working basis.





Developmental Cycle of Benign Tertian Parasite.

FIG. 1. Very early form of parasite, showing chromatin granule, "milky zone," and spheroidal body.

FIGS. 2, 3. Typical young ring-shaped parasites.

FIGS. 4, 5. Subdivision of chromatin, development of body and appearance of pigment in later ring-forms.

FIG. 6. Double rings, in single parasite.

FIGS. 7, 8. Turban-shaped parasites. Secondary rings, eccentric position of chromatin.

FIG. 9. Double infection of cell.

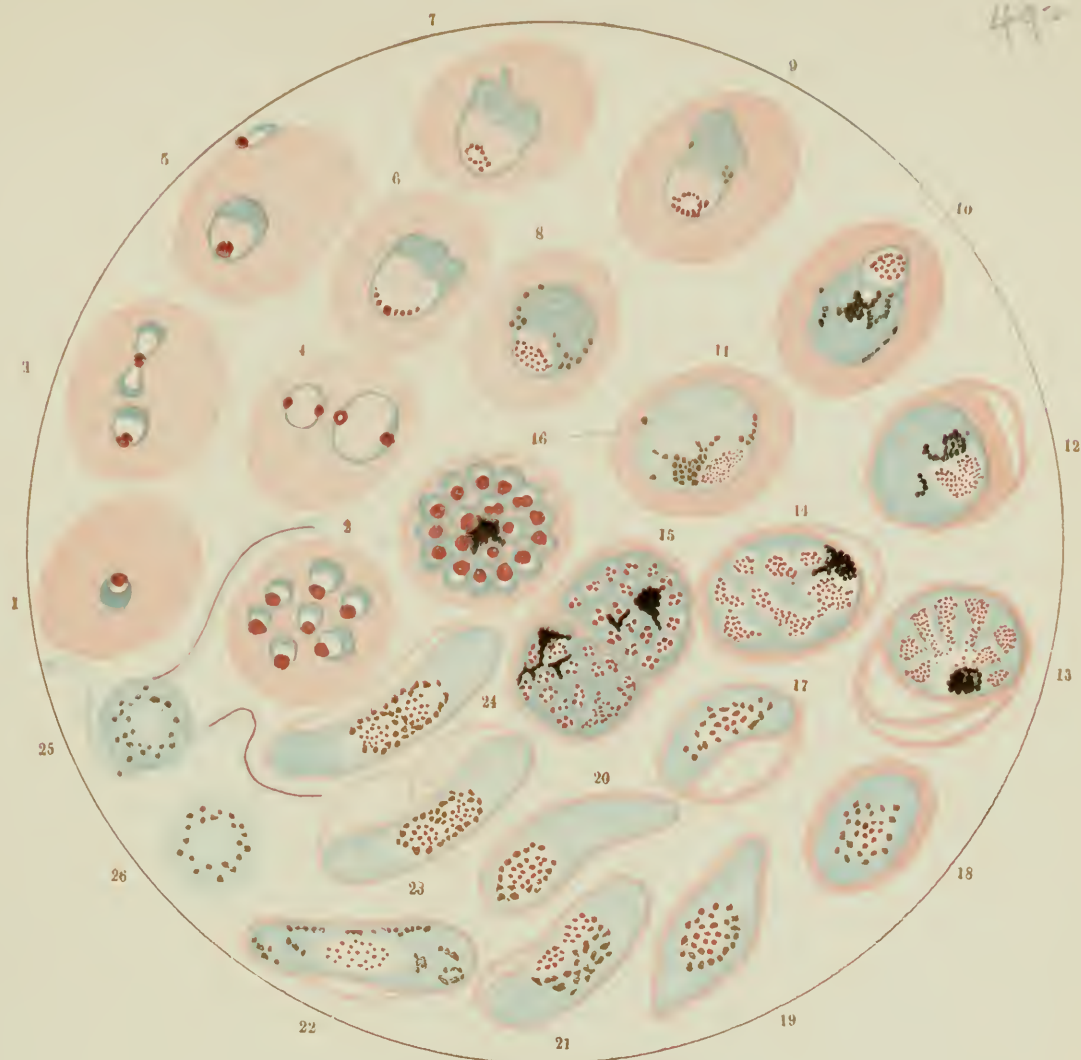
FIGS. 10, 11. Complex amœboid figures in doubly infected cells.

FIG. 12. Full grown form, with large eccentric nucleus.

FIGS. 13, 14. Protrusion of chromatin granules and milky substance in body of full-grown parasite.

FIGS. 15, 16. Division of chromatin granules into groups in reticulated presegmenting bodies.

FIG. 17. Tertian rosette.



Cycles of Aestivo-autumnal Parasite.

FIG. 1. Very young form.

FIG. 2. Infection of one cell with seven young parasites. (Drawn from a marrow-smear.)

FIG. 3. Triple infection. Two parasites joined by single chromatin mass.

FIG. 4. Double infection. Peculiar rings with two chromatin grains at opposite poles.

FIG. 5. Double infection. Small ring adherent to cell.

FIGS. 6, 7. Signet-ring forms. Sub-division of chromatin.

FIGS. 8, 9. Later ring forms, with sub-divided chromatin and few pigment grains.

FIGS. 10-12. Full-grown forms with finely sub-divided chromatin and gradual concentration of pigment.

FIGS. 13, 14. Stages of presegmenting forms, with concentrated eccentric pigment.

FIG. 15. Double infection with separate presegmenting bodies.

FIG. 16. Aestivo-autumnal rosette.

FIGS. 17, 18. Young crescent and ovoid.

FIG. 19. "Pulsating" crescent.

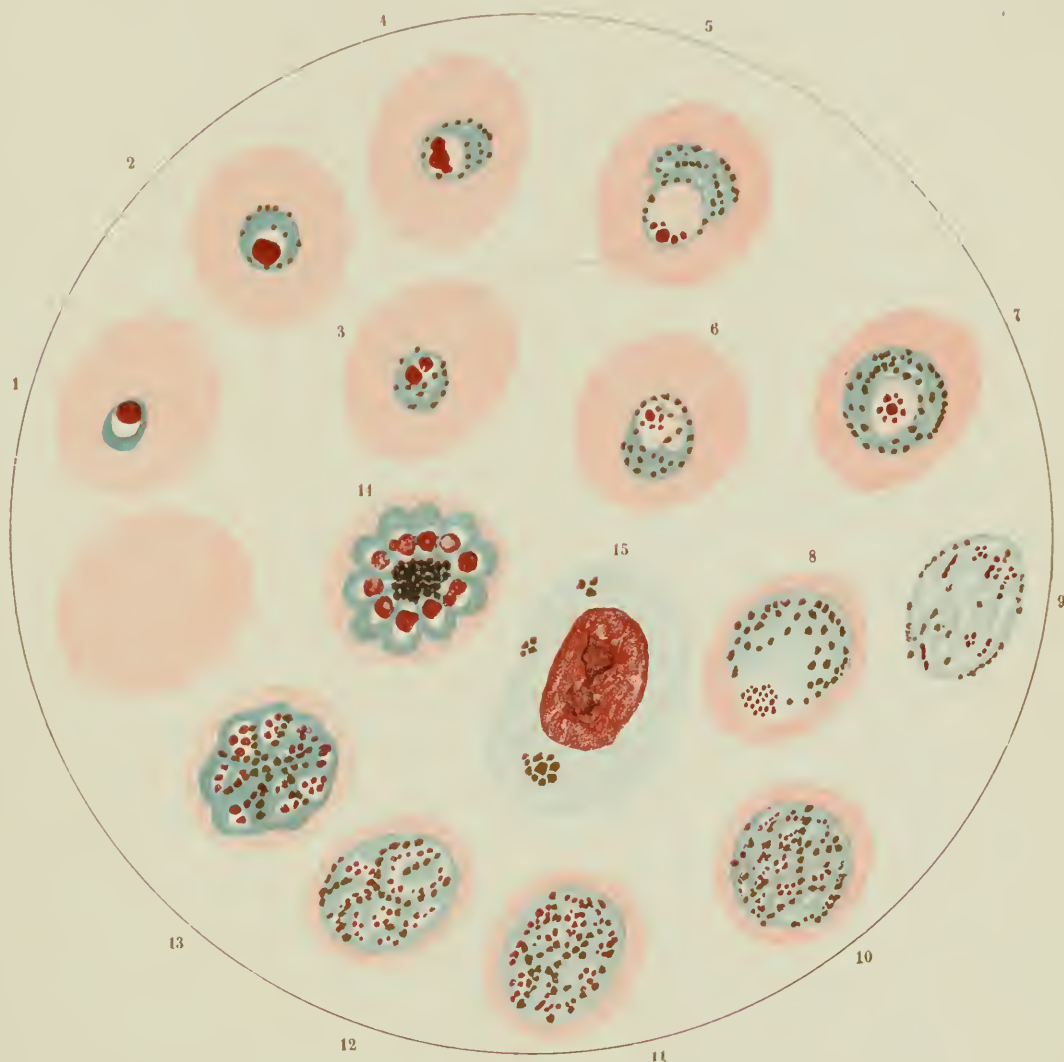
FIGS. 20-22. Various forms of crescents.

FIG. 23. Two bows about single crescent.

FIG. 24. Finely developed crescent; two masses of chromatin; achromatic substance; double wreaths of pigment.

FIG. 25. Diagrammatic flagellating body.

FIG. 26. Extra-cellular sterile body.



Cycle of Quartan Parasite.

FIG. 1. Very early non-pigmented form.

FIGS. 2, 3, 4. Small quartan rings, with large chromatin masses and abundance of pigment.

FIG. 5. Turban-shaped ring, with subdivided chromatin.

FIG. 6. Subdivision of ring and of chromatin granules.

FIG. 7. Coarse quartan ring with central chromatin granules.

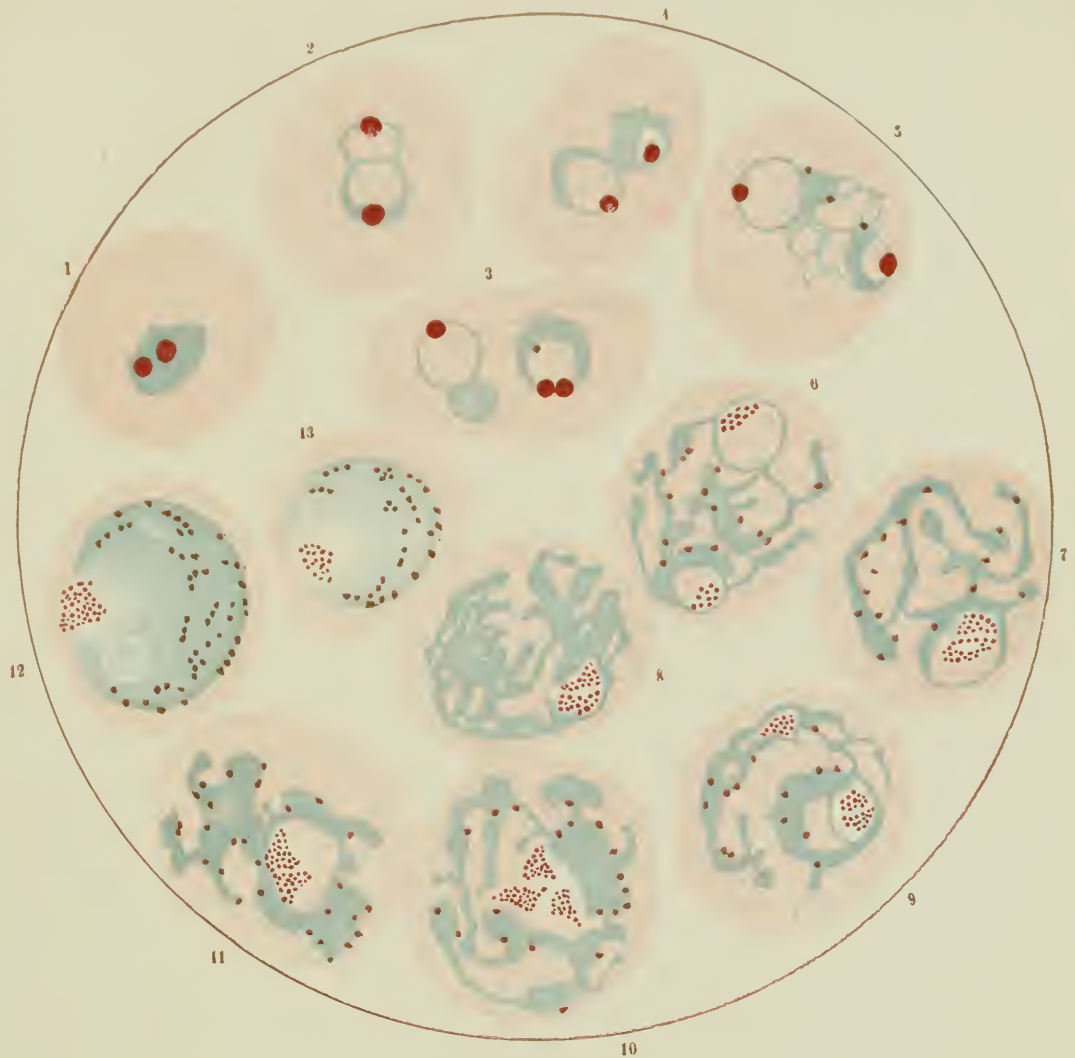
FIG. 8. Full-grown quartan parasite, with eccentric chromatin, hyaline body, and abundance of pigment.

FIG. 9. Extra-cellular reticulated body.

FIGS. 10-13. Quartan presegmenting forms.

FIG. 14. Quartan rosette.

FIG. 15. Pigmented mononuclear leucocyte.



Conjugating Cycle of Tertian Malarial Parasite.

FIG. 1. Single compact body with double chromatin masses.

FIG. 2. Conjugating rings of unequal size.

FIG. 3. Double infection with a coarse ring, double chromatin granules, and a thin ring form.

FIGS. 4, 5. Early stages of conjugation of a thin ring and a compact body.

FIG. 6. Early amœboid figures of conjugating rings.

FIG. 7. Double nuclei in amœboid parasite.

FIG. 8. Union of nuclei, and subsidence of amœboid motion in older conjugating parasites.

FIGS. 9, 10. Stages of union of bodies and of three chromatin masses, of two conjugating parasites.

FIG. 11. Complete union of bodies and nuclei.

FIGS. 12, 13. Comparative sizes of full-grown forms developed with and without conjugation.

THE NERVES OF THE CAPILLARIES, WITH REMARKS ON NERVE-ENDINGS IN MUSCLE.

A NEW THEORY OF LYMPH-FORMATION AND OF GLANDULAR SECRETION.

BY CHR. SIHLER, PH. D., M. D., CLEVELAND, OHIO.

Taking leave of the Johns Hopkins University in 1880, where I had spent three of the most satisfactory years of my life, I set myself the task to investigate the submaxillary gland in order to find the terminations of the chorda tympani. The reason for this was the importance of that nerve to physiology. To those not interested particularly in physiological questions, I may say that the chorda tympani may be looked upon as the key to solve the question of the act of glandular secretion as well as of lymph-formation in general; and as the anatomical arrangements of the submaxillary gland and its nerves are such that physiologists can undertake experiments on these nerves with very clear and lucid results, it is of course very desirable that it should be clearly understood which tissues are really influenced and made to act when the chorda is subjected to electric stimulation.

As nineteen years have now passed by since I attacked this problem, and as I have devoted all my spare time—so far as original investigation is concerned—to this and allied questions, I consider it not out of place to report my results as briefly as possible, particularly as my investigations have brought out facts which have not found a place in our anatomico-physiological conceptions, and as the views which I have been forced to adopt are by no means in harmony with the histological and physiological teaching of the present day.

Of one working on the nerve-endings of the chorda, it will very properly be expected that he make himself familiar with the endings of other motor nerves, particularly those in muscle; and this I have done in the hope of finding here histological facts which would throw some light on the nerve supply of the gland. In muscle tissue we

find three kinds of nerves: those going to the muscle spindles; the motor nerves proper ending on the muscle fibre; and the nerves going to the capillaries and other blood-vessels. The last-mentioned ones interest us directly, the motor ones indirectly, and these will be touched upon later. The former can be found by treating frog's muscle according to the acetic-acid-haematoxylin method which I described some years ago,¹ and which is published in Boehm and Oppel's handbook.²

Teasing out muscular tissue, one finds among the motor nerve fibres fine non-medullated fibres, which can be traced to the capillaries. The nerves running alongside of the capillaries can also readily be found with medium powers, but these are not the terminal branches. With the immersion lens there can be seen attached to the capillary wall some still finer nerve fibres, which pass off from the fibres just mentioned. These also have their nuclei and differ from nerves running alongside of the capillaries by their small caliber and their varicosities. They are so intimately attached that it is not easy to trace any fibre a long distance, but their nuclei always reveal them.

Although L. Bremer³ pointed out these nerves some time ago, and although his description of them, so far as their connection with the capillary vessels and their appearance is concerned, agrees with my own observation, these nerves have not found a place in our histological reasonings, and perhaps it is just as well that they have not—at least on the strength of Bremer's work, because his other statements about them I consider erroneous. Kölliker, in his "*Handbuch der Gewebelehre*" (6th edition), in the paragraph on the sensory and vascular nerves of muscles, discusses these nerves in the sternocutaneous muscle of the frog, but adds that regarding their origin and destination he has not been able to form any definite opinion. This illustrates perhaps the state of our knowledge about them. Bremer, it is true, claims that they are branches of the motor nerves proper,

¹ *Cleveland Medical Gazette*, 1894-5, x, p. 255; and *Arch. f. Physiol.*, 1895, p. 202. The method is described also in the *Zeitschr. f. wiss. Zoologie*, 1900, lxviii, p. 323.

² Boehm and Oppel. *Taschenbuch der mikroskopischen Technik*. München, 1896.

³ *Arch. f. mikr. Anat.*, 1882, xxi, p. 663.

but I have always found that, where I discovered such fine nerve fibres free amongst muscle fibres, I could find hidden away, amongst the motor fibres, some fine non-medullated fibrils, from which they took their origin, and I have never seen them branch off from a motor nerve, although one can readily understand how Bremer came to make this statement, so deceptive are the appearances in many instances.

These nerves, surrounding the capillaries, come from a special set or class of nerve fibres, whose appearance would lead one to consider them as belonging to the vasomotor system.

Further, in regard to their connections in the periphery, I disagree also with Bremer, who says that, aside from connection with the motor nerves, they observe a strict seclusion, never leaving the vessel. Specimens from the frog's tongue show that these nerves form connections with the sensory nerves, as the nerve fibres from the papillæ can be seen to pass into the network of nerves surrounding the vessels. This fact would lead us to think of them as sensory nerves.

Lastly, if the nerves surrounding a capillary are traced towards the centre, they can be seen to pass into the plexus surrounding the larger vessels. Very frequently two fibres can be found running on the walls of the capillaries, and if one compares the nerve supply of striped muscle with that of the capillaries it can be seen that the latter are far more richly supplied with nerves than is striped muscle.

Tracing the fibres of the chorda tympani in the submaxillary gland, I came to the conclusion that the gland cells themselves are not supplied with nerve fibres, but that the terminal fibres are found on the capillary vessels, just as in the case of the capillaries of muscular tissue, and that, therefore, those nerves of muscle which are analogous to the glandular nerves are not the motor nerves proper, but are those going to the capillaries.

The important fact which I wish to point out in this connection is that, inasmuch as the chorda is considered universally as a motor nerve, and if I am right in placing the nerve terminations of the chorda on the capillaries, it would be established that the capillary nerves are fed by motor trunks and that their function is of a motor character.

To sum up the histological facts, then, there exists a vast peripheral network of fine nerves coëxtensive with the capillaries of the muscles and glands, which has connection with sensory nerves and into which motor-nerve trunks also enter, and which I therefore look upon as being sensory and motor at the same time. Whether there is a distinction here between sensory and motor fibres, or whether any single fibre may be both, does not concern us. I have no difficulty in imagining the latter to be the case, as every neurone, whether it be called sensory or motor, both receives and imparts impressions. We ought not without proof to apply conceptions pertaining to the well-selected strands of fibres in the spinal cord to a peripheral plexus where direction has little to say. The existence of such a plexus would not support the hypothesis that the entire nervous system is made up of independent neurones.

What is the significance of these nerves of the capillaries, what is their function? Although I am not so fortunate as to have at my command a laboratory in which I could experiment on these nerves, yet if we take into consideration the histological facts together with certain clinical observations and physiological experiments which have been made on these nerves, although without any accurate anatomical knowledge about them, we have, I think, satisfactory evidence and scientific support for the hypothesis which I shall here state briefly: These nerves, so intimately connected with the capillaries, influence the protoplasm of their walls in such a way that, according to the activity of the nerves, the transudation of lymph is increased or diminished. Further, they take cognizance of local disturbances of a chemical or mechanical nature, and in response to local causes of irritation influence the capillaries of a part to pour out more fluid and act in the interest of the organ in question. As increase of lymph-formation and vasodilatation must, in the long run, go hand in hand, it would seem reasonable to suppose that the nerve fibres going from the capillaries to the arteries and veins may exert an inhibitory influence on the vasoconstrictors, or a stimulating one on the vasodilators, whereby a larger supply of blood is furnished to the irritated part.

I will now enumerate the reasons which give support to the hypothesis mentioned. I must premise, however, by way of explanation, that when I speak of increased lymph-production by the capillaries, I consider that this is brought about not by simple increase in the caliber of the vessel, but by some specific action of the cells of the capillary wall—an activity as specific for these cells as contraction is for muscular tissue.

(1) The first reason offered in support of my hypothesis is based upon our knowledge of the physiological properties of the capillaries as manifested in processes of transudation. Michael Foster in discussing the problem of lymph-formation says that “the condition of the vascular wall so profoundly influences the transit of material as to render the process very complex. We may probably regard it as too complex to be compared even with filtration through a filter capable of widely changing in texture from time to time and as more nearly resembling the process of secretion.” My views are in exact accordance with these words of this distinguished physiologist.

In the familiar facts of muscular contraction we have unmistakable evidence that it is possible for living tissue to experience the most profound molecular changes through the influence of nervous action. Why should this not be possible for other tissues? That very marked changes can take place in the capillary wall is evident from a consideration of what occurs during glandular activity and during the process of inflammation.

However glandular secretion may be brought about, it would seem impossible that the capillary wall should be wholly indifferent while the saliva and other secretions are poured out from the ducts so promptly and abundantly. There is sufficient physiological, as well as pathological, evidence to indicate that we cannot look upon the capillary wall as containing physical pores open to suction from the gland cells and to the direct action of the blood-pressure. The vital condition of the capillary wall must be different when secretion is going on from that when this is not the case.

Further, when inflammation exists, not only are larger quantities of lymph exuded through the walls of the capillaries, but even cor-

puscular elements find their way through these structures; and yet after the pathological process has run its course, the capillaries return to their original state, thus illustrating what great changes can take place in the cells of the walls of the smallest blood-vessels.

(2) A second support of the hypothesis is to be found in the histological facts mentioned—the enormously rich supply of nerves to the capillaries. These must have their purpose, and, if my view that the chorda tympani ends on the capillaries should be confirmed, scarcely any further proof of the hypothesis would be required. Although my observations have been limited to muscular tissue and the salivary glands, these two tissues are sufficiently representative to make it probable that the capillaries in other organs are supplied with nerves of a like character.

(3) When the chorda tympani is stimulated the saliva appears in Wharton's duct under a pressure which may be greater than that in the carotid artery; atropia stops the secretion even if, through nerve stimulation, the vessels remain dilated; and even after cutting off the blood-supply, saliva is said to be secreted.

To explain these well-known facts, simple vasodilatation being insufficient, physiologists have called into service the gland cells themselves and have ascribed to them nerves which are supposed to incite the gland cells to secretion just as stimulation of the motor nerves causes muscular contraction. The internal changes, however, in muscle during contraction are widely different from those in active glands, whose secretion is composed mostly of water.

Let us analyze this assumption of the physiologists. Let us assume that the gland cells are provided with nerves and that some internal changes go on in these cells in consequence of their stimulation. How can these changes influence the capillary wall, as no nerves pass from the gland cell to this wall, and as in the capillary there are no open pores through which a sucking action could be carried on? Upon this assumption I see no other way by which the capillary could be influenced to produce more lymph than by supposing that it is sensitive to the state of concentration of its surrounding lymph, and that greater concentration of this will induce the capillary to pour out more fluid.

To my mind the gland cells are not constructed to furnish large quantities of fluid, they are not in immediate contact with the source of supply, while the capillary cell, with its flat surface and thin walls, continually bathed with serum on its inner surface, would seem to be made for such a purpose. I cannot understand why gland cells should have their own nerves, while an independent action of these cells manifested in the formation of chemical substances, without the production of fluid to wash away and dissolve them, would be of no use to the organism. On the contrary, I can see that, if provision were made to have large quantities of lymph poured out over the gland cells, that this influence would act as a sufficient stimulation to the gland cell to transform some of its reserve material into soluble matter and to push on the fluid received from the capillaries. As the water in glandular secretions is to be accounted for as well as the dissolved substances, as a separate nerve supply to the gland cells seems to be physiologically unnecessary, and as I have satisfied myself of the existence, both in muscular tissue and in the salivary glands, of a rich supply of nerves to the capillaries, I cannot without better evidence than we now possess admit the reasoning of the physiologists on this subject as sound.

(4) The fourth point relates to the results of experiments on the branch of the chorda going to the tongue. The chorda tympani coming from the skull divides into two parts, one going to the sub-maxillary gland, the other to the tongue.

In Cohnheim's "Pathology,"⁴ Ostroumoff's experiment is mentioned and discussed, and as I consider it important and interesting, I shall introduce it here:

"If," Cohnheim says, "the peripheral stump of the dog's cut lingual nerve is stimulated for a period of time by induction currents of increasing strength, the rapidly forming intense hyperæmia of the corresponding half of the tongue is accompanied by a pronounced oedema, which, in about ten minutes after the beginning of the stimulation, is evident to the naked eye, and in the next ten minutes increases uninterruptedly to a very considerable size. This very remarkable experiment,

⁴ Vorlesungen über allgem. Pathologie, Bd. i, p. 134. Berlin, 1882.

about which I have been kindly informed by M. Ostroumoff, is indeed calculated to raise doubts whether all congestions, however brought about, are of equal significance so far as lymph-formation is concerned, and the more so as there are certain observations on man, for example, the rapid formation of urticaria wheals, due to undoubted nervous influence, which point only too clearly to the *existence of intimate relations between the formation of lymph and the innervation of vessels.*" (The italics are my own.)

We have evidence, then, that stimulation of the branch of the chorda going to the submaxillary gland results in the production of fluid contained in Wharton's duct and that stimulation of the branch of the chorda going to the tongue likewise produces fluid, which is here contained in the lymph spaces of the tongue. Now it might be expected that the formation of lymph by the different branches of the same nerve, called forth by the same agency, would be explained on similar physiological principles as brought about by similar histological elements. But no; in the case of the gland, while the vessels are allowed to supply the organ with more blood, the gland cells are summoned in order to explain the occurrence of the fluid in the duct; while in the case of the tongue an ill-defined vasomotor action has to account for the accumulation of fluid in the lymph spaces.

If it is considered possible that, in consequence of the stimulation of gland cells by *hypothetical* nerves, these cells can not only form mucus, but produce from somewhere large quantities of fluids, why, I would ask, may it not be assumed that nerves that *can be demonstrated* can incite the cells of the capillary walls to increased transudation of lymph, which passing to the gland cells will induce them to add their chemical product to it, while retaining the albuminous matter, and to pass both fluid and product on into the ducts. This hypothesis demands no new conceptions, but merely a transference of accepted physiological doctrines from the gland cells and their problematical nerves to the cells of the capillaries and their demonstrated nerves. Ostroumoff's experiment on the chorda branch going to the tongue demonstrates plainly that the increased flow of fluid from the vessels into the tissues can be accounted for without assuming the participation of gland cells.

(5) Further support for the views here advocated can be found in experiments made by Rogowicz⁵ under Heidenhain's direction to clear up the connection between vasodilatation and lymph-formation. He shows by actual measurements that all forms of dilatation are followed by an increased flow of lymph. This fact does not interest us here particularly, as this increase might be explained simply by the larger blood-supply to the part. Another experiment, however, which he instituted in order to get an insight into the promptness with which an increase in lymph-transudation is brought about by stimulation of the chorda of the tongue, is of more interest to us. "While," says Rogowicz, "the lingualis was being stimulated, a saturated solution of sulph-indigo-carmin was injected into the saphenous vein. The aspect of the tongue under these circumstances is most remarkable, while the circulation of the blood on the side of the tongue not stimulated is yet in a normal condition. The half of the tongue corresponding to the stimulated lingual nerve takes on a deep blue color in a very short time, and that, too, at a point of time when the other side presents only a pale blue hue. If the injection is now discontinued this difference in coloring will continue for quite a while. It depends on a more rapid filtration of lymph into the tissues of the stimulated half of the tongue." Rogowicz then explains that the blue color is not due to the coloration of the blood, which remains red, but that the coloring matter is in the connective-tissue spaces throughout the tongue, as can be seen in transverse section through the hardened tissue. This very prompt and energetic lymph-formation could hardly be explained by simple dilatation of the arteries.

Of further importance to us here are other facts brought out in the course of these experiments. Rogowicz found that after injection of curare the production of lymph may be increased three to four times, without any apparent increase of the blood-supply, as, for example, from 35 cc. to 115 cc., and from 81 cc. to 240 cc. We thus see that with a stationary blood-supply lymph-formation can be increased. On the other hand, the experiments on the submaxillary gland have shown that atropia will prevent the formation of lymph even if vasodilatation is brought about by nervous influence.

⁵ Pflüger's *Archiv*, 1885, xxxvi, p. 252.

(6) Heidenhain⁶ in his researches on the pseudomotor contraction of the tongue has made some observations which speak strongly in favor of the view defended in this paper. Searching for the cause of the contraction of the tongue, the muscle fibres of which had been made hyperæsthetic through section of the hypoglossal nerve some days previously, he found that stimulation of the chorda, which under these conditions causes contraction of the lingual muscles, was always followed by hyperæmia of the tongue. Heidenhain's surmise that the immediate stimulus to the muscle contraction is an increased flow of lymph was supported by the subsequent experiments of Rogowicz made under his direction. Under the conditions here present even physiological salt solution, towards which normal muscle is indifferent, will produce active muscular contractions.

The two facts, however, which are of most importance to us are that, when Heidenhain had clamped the arteries supplying the tongue, stimulation of the chorda still produced contractions, though of diminished force, as might be expected, and only for a short time; and that these contractions would take place upon stimulation of the chorda before any visible hyperæmia, indicating a pouring out of lymph from the capillaries, had set in. Heidenhain says in closing the discussion:

"After these observations it does not seem improbable that a rapid increase in the amount of fluid saturating the tongue may be looked upon as the cause of the contractions of the paralyzed muscle. But I must lay particular stress on the point, that I have not offered convincing proof for this assumption. . . . Above all, the evidence is lacking that even after interruption of the circulation in the tongue stimulation of the chorda can produce a transudation of lymph."

Regarding this last statement, it seems to me that the occurrence of muscular contractions due to stimulation of the chorda furnishes the evidence for lymph-formation through action of the capillaries themselves. When the arteries are clamped, the chorda can influence nothing else but the capillaries, and as it has been shown, on the one

⁶ *Arch. f. Physiologie*, Suppl.-Bd., 1883, p. 133, and Rogowicz, *Pflüger's Archiv*, 1885, xxxvi, p. 1.

hand, that lymph-formation can be induced without increase of blood-supply; and, on the other hand, that these degenerating muscles of the tongue respond even to such feeble stimulation as injections of normal salt, it seems justifiable to regard these contractions as an evidence for lymph-transudation brought about by activity of the capillaries.

At any rate, the proof for this function of the capillaries is a good deal stronger than that now considered sufficient to explain the production of the watery parts of the saliva by action of the gland cells and their hypothetical nerves. When the high pressure in Wharton's duct, the action of atropia, the secretion of saliva after severance of the head, are adduced to demonstrate the activity of the gland cells, it must not be forgotten that not only the gland cells and their hypothetical nerves, but also the capillaries and their real nerves, should be taken into account as factors in the phenomena. In the case, however, of these contractions of degenerating muscles in the tongue, with arteries clamped and chorda stimulated, we have to do solely with the capillaries and their nerves. We can exclude everything else. No one has ever separated under analogous circumstances the gland cells in order to see what function they can then perform.

(7) Attention may be called also to injuries of the cornea by a foreign body or other irritant, as throwing light on the character of our nerves. When the fundamental experiment of Cohnheim is carried out on the mesentery and the changes in the capillary walls are observed, these are at present usually explained by the injury directly inflicted on the capillaries. But when a piece of steel in the centre of the cornea sets up not only a violent injection of the conjunctival vessels, but also an increased flow of lymph and an emigration of white corpuscles, we cannot explain these phenomena by a direct injury to the vessels. If, as generally believed, a change in the walls of the capillaries is necessary to explain the emigration of white blood-corpuscles in inflammation, then we have here an illustration of the influence of sensory nerves upon the nerves of the capillaries, and not solely such an influence upon those of the arteries and veins.

(8) Heidenhain, who is the main authority for the prevailing

theory of secretion, which imposes upon the gland cells the function of drawing the necessary amount of fluid from the blood, and passing it into the duct, points out an apparently very serious objection to this theory in the œdema which takes place when, the ducts of the sub-maxillary being tied, the chorda is stimulated. He says: ⁷

“This phenomenon seemed to be irreconcilable with any form of attraction-hypothesis, and to be in favor of a propelling force for the stream of fluid, a force independent of the gland cells and set into action by nervous influence. But there is one explanation which had previously escaped me in spite of all my efforts to find one. As soon as the œdema begins to develop the tension of the connective-tissue capsule surrounding the gland increases to a high degree, which must have the effect of compressing the veins which pass through the capsule. Thus the artificial filtration-œdema causes, through the difficulty it establishes for the escape of blood from the gland, an œdema due to passive venous congestion (*Stauungsödem*), which becomes greater as the stimulation of the chorda increases the blood-pressure in the capillaries.”

As bearing upon Heidenhain's explanation, the following questions seem pertinent:

(a) How is that part of the œdema explained which sets up such a very marked tension in the capsule that it shuts off the circulation? Would not this interfere with the whole process of secretion when little or no fresh blood can pass through the gland?

(b) What evidence is there for such constriction of the veins?

(c) Would not the gland cells, as soon as they found an impediment to the passing on of the water which they had taken out of the blood current, cease their activity, so that such an œdema would not take place?

(d) Would it not be more satisfactory to explain this œdema in the gland in the same way as Cohnheim and Ostroumoff explained the œdema of the tongue after stimulation of the chorda branch, where there is no impediment to the outflow of blood through the veins?

Heidenhain and other physiologists have, so far as I am aware, never considered nor mentioned the theory which we propose, and I think for several reasons:

⁷ Hermann's *Handb. d. Physiologie*, Bd. v, Theil i, p. 77. Leipzig, 1883.

First. Physiologists have been so impressed with the analogy of glandular secretion to muscular contraction that they have assumed without sufficient evidence that motor nerves of glands exist and excite the gland cells to activity.

Second. It has not heretofore been established that the capillaries are richly supplied with nerves and that these are of a motor character.

Third. In speaking of vasomotor activity, the functions of the larger vessels and those of the capillaries have not been clearly distinguished from each other.

While the facts and arguments which I have thus far considered seem to me to speak strongly for an independent activity of the capillaries under the control of the nervous system, the decisive point to be established is whether the chorda terminates in the submaxillary gland on the capillaries and not in the gland cells. The establishment of this point would outweigh in importance all of the other considerations adduced, as the chorda tympani is acknowledged to possess motor characters. It is, therefore, important to give shortly some reasons why I believe that the chorda tympani terminates on the capillaries.

In this connection, as will appear, a true knowledge of the motor nerve endings in muscle is of importance. The present teaching about motor endings in muscle is that the end fibrils penetrate the sarcolemma and as naked end-fibrils come into contact with the muscle substance. My investigations have led me to a different conclusion. I find that the motor endings remain on the outside of the sarcolemma, and, except at the surfaces, where muscle and nerve come into contact, are covered with the strong sheath of Schwann which has its own nuclei. What may be the exact condition of things at the points where muscle and nerve fibre are in actual contact—whether the sarcolemma and neurilemma are wanting there, or perforations exist, or whether electrical phenomena observed in nervous activity can be used to explain the processes going on there—I cannot say. The precise relation of muscle to nerve here is an unsolved and difficult histological problem.

It would lead too far to present here in full the evidence in support

of my views concerning these nerve-endings. Much of it I have brought forward in other papers,⁸ but a few points may be here adduced. The important fact is that these motor endings have their own nuclei. Formerly these nuclei gave no trouble to the advocates of the so-called hypolemmal theory, as one could consider them as belonging to the axis cylinder. But now when we know that the axis cylinder is a process of a cell in the brain, spinal cord or one of the ganglia, and that this process possesses no nuclei of its own, we must ascribe the nuclei in the nerve-endings in muscle to the sheath of Schwann, and why should we not? They agree in appearance with the nuclei of other fine nerves, where there is no question but that the nuclei belong to the sheath of Schwann. But if this sheath is continued over the end fibrils, its presence would prevent direct contact between nerve substance and muscle, even if the endings were placed beneath the sarcolemma.

Lately, it is true, Huber and De Witt⁹ have announced that these end fibrils are devoid of nuclei, although Kühne and Kölliker have described them. The advocates of the hypolemmal theory should rejoice at this discovery, inasmuch as the presence of these nuclei would deal the death-blow to that theory. I cannot here enter into a criticism of this observation of Huber and De Witt, and will only say that I have seen these nuclei attached to the end fibrils in hundreds of perfect specimens and that I consider the evidence advanced by these investigators as inconclusive and their methods as inadequate to settle this point.

Further evidence for the epilemmal position of the end fibrils is furnished by a class of nerve endings in the frog's muscle, not described in the text-books. Here we find not only the form described in the text-books, but also a variety of other endings. There are terminal branches of non-medullated nerve fibres, which, after attaching themselves to a muscular fibre for a little distance, form an arch quite free from the fibre, again come into contact with the muscle and then, perhaps, form a second arch, and so on. The nuclei may be found

⁸*Arch. f. mikr. Anat.*, 1895, xlv, p. 709; 1900, lvi, p. 334, and *Zeitschr. f. wiss. Zoologie*, 1900, lxxviii, p. 323.

⁹*Journal of Comparative Neurology*, 1897-8, vii, p. 169

as well on the free arch as on the attached portion of the nerve fibre. There are other forms where these arches grow smaller, but the festooned arrangement can still be recognized. Now these arches, whether large or small, show clearly where the end fibre is situated, for it is impossible to place them under the sarcolemma, and it would be an unwarrantable assumption to suppose that in one case the nuclei and end fibrils are above the sarcolemma, and in the other under it. The forms with small arches have been misunderstood both by Kühne and by Bremer. Kühne described them as artefacts due to the gold method, and at the same time inconsistently spoke of them as a special class of atypical endings occurring more frequently in certain parts of the body than elsewhere.

The hypolemmal theory was announced many years ago when the methods were very imperfect, although they yielded valuable results. I would here point out that a figure in Stricker's Handbook,¹⁰ which has been copied into many text-books, is erroneously interpreted. The nuclei which are ascribed to the sheath of Schwann are really those of Henle's sheath, so that according to this figure Henle's sheath is made to pass with the terminal fibres through the sarcolemma. If the methods of that day failed to distinguish such an important point as this, is it likely that they sufficed to determine such a delicate and subtle matter as the precise relation of the end fibrils to the sarcolemma? It is true that Kühne says that in profile views the passing of the sheath of Schwann into the sarcolemma can be observed without difficulty, but I have examined hundreds of good specimens and have never been able to convince myself of this, while, on the contrary, I have seen many facts inconsistent with this view. I have shown in recent papers¹¹ that in the frog and in the small muscle fibres of the snake Henle's sheath is open at the end, thus allowing the cerebrospinal fluid to escape, and that in other animals this sheath extends over the end fibres and assists in forming Kühne's "Sohlen-substanz."

These ivy-like forms of nerve-ending in the frog further show

¹⁰ Stricker's Handb. d. Lehre von. d. Geweben, Bd. i, p. 154, Fig. 35. Leipzig, 1871.

¹¹ *Arch. f. mikr. Anat.*, 1900, lvi, p. 350, and *Zeitschr. f. wiss. Zoologie*, 1900, lxxviii, p. 323.

that nerve fibres may influence muscle not only at the places where they actually terminate, but also at places where they merely come into contact with the muscle, the fibre itself passing on to a second muscle fibre and so on. This view of the motor nerve-endings in striped muscle is of general importance, as it indicates that in other situations cells may be influenced by similar contact with nerves without the presence of naked end fibrils. I therefore consider the plexus of fine nerves found in smooth muscle and built like certain motor endings in striped muscle, as the "nerve-endings" of this class of muscle, and consider it futile to look for a nerve-ending for each plain muscle-fibre cell.

To return to the submaxillary gland, I consider that there are sufficient analogies to justify my interpretation of the nerves which I have found there in connection with the capillaries, as the terminations in this organ of the chorda tympani, for there is agreement in histological details between these nerves and nerve terminations in plain and striped muscle, as well as the nerves of the capillaries in muscle. If one stains with Beale's carmine a small but sufficiently large piece of the gland to contain all the structures, and presses it out carefully, among the various nuclei four kinds can be distinguished: those of the gland cells, those of endothelial cells of the capsule, those belonging to the capillaries, and other nuclei just outside of the capillaries; these last are the nuclei of the nerve fibres clinging to the capillary wall. While I am able to demonstrate nerves going to the capillaries, I have failed to find nerves going to the gland cells. I am aware that Dogiel,¹² Huber¹³ and others have reported finding nerve endings on the cells of certain glands, as the lachrymal and submaxillary. The correct interpretation, however, of the pictures presented in specimens treated by the methods employed by these observers seems to me to be still doubtful, and in view of my negative results on this point I am not prepared to accept their conclusions.

Since my first publication, over fifteen years ago, on the structure and nerves of the submaxillary gland, and of muscle,¹⁴ the conclusions

¹²*Arch. f. mikr. Anat.*, 1893, xiii, p. 632.

¹³*Journal of Experimental Medicine*, 1896, i, p. 281.

¹⁴Studies from the Biological Laboratory of the Johns Hopkins University, 1884, iii, p. 155. *Cleveland Medical Gazette*, 1885-6, i, p. 193.

then formed have been confirmed by the application of my new method which shows that the nerve supply of the capillaries is rich enough to be looked upon as the termination of the chorda. Still further confirmation of my views has been afforded by study of the capillaries of muscle. At that time I advanced the theory of the independent activity of the capillary wall and its importance in the production of lymph, and suggested that these real nerves explain all the experimental facts fully as well as do the hypothetical ones on the gland cells.

Although I have never seen the theory which I propose in this paper offered before, it seems to have been foreshadowed by Cohnheim, as will appear from the following extracts from his "Pathology." I have italicized certain passages to which I wish to call special attention. Discussing the theory of inflammation, Cohnheim¹⁵ says:

"But if it is impossible at the present day to give a more accurate definition of this alteration [that in the walls of the blood-vessels during inflammation] no blame can be attached to us, the pathologists, on account of the obscurity in which even the normal process of transudation is at present involved. Nobody can assign the cause for the different chemical composition of the transudates in the different regions of the body; nobody knows with certainty on what condition it depends, that the vessels of the pleura and pericardium secrete a transudate which is so much more concentrated than that of the vessels which furnish the cerebrospinal fluid. Every day some new, astonishing evidence presents itself showing, on the one hand, what an important influence the walls of the blood-vessels exert on the vital functions and, on the other, how energetically they react to all sorts of injuries. That through their activity the caliber of the vessels can be increased or diminished is a well-known fact, and has been taken into account both by physiologists and pathologists. But since it has been shown in Ludwig's laboratory that curarized animals produce lymph of higher concentration than the normal, *does not this fact point to relations of a quite different character between the vascular walls and the blood-current?* If the skin of a dog assumes a red color after an injection of curare into a vein, we are not very much astonished, as we are accustomed to ascribe to curare a paralyzing or a stimulating influence on the vascular nerves. Through this

¹⁵ Op. cit., i, p. 282.

well-known action, curare cannot, however, exert an influence on transudation; but why should it not be able to act on other constituents of the vascular wall than solely the nerves of the muscle-fibres? Why not directly on those that are instrumental in the process of transudation, even if these are as yet unknown to us? *It is possible that nerves come into play here or that nerves can exert at least an important influence on the process of transudation*, as has been long established for a number of secretions and recently also for the blood-vessels of the tongue by Ostroumoff's experiment. Further striking evidence for the view that *the nervous system can influence the qualities of the vascular walls in a quite different way than by simply acting on their contractility* is furnished by the rapid onset of œdema in the paralyzed extremities, repeatedly observed in cases of acute myelitis, and also by Gergens' demonstration that the blood-vessels of frogs whose spinal cord has been destroyed, allow much larger quantities of fluids and even pigment granules to pass through their walls than is the case with frogs with normal medulla. Who would venture to decide in the present day whether or not such *specific nervous arrangements situated in the vascular walls themselves* come into play also in the process of inflammation?"

It is evident from this quotation that Cohnheim recognized the inadequacy of limiting the influence of nerves upon blood-vessels merely to an effect upon the caliber of the vessels, and that he brought forward strong arguments for the view that the nerves must be capable of exerting also other influences upon the vascular walls, especially in the production of lymph and in inflammatory processes. I have, therefore, great satisfaction in having pointed out, and, I hope, correctly described those nerves which Cohnheim saw with his mental vision, and I think I may say, without assuming too much, that, rightly interpreted, there is here entire harmony between physiological theory and anatomical facts.

It may have occurred to the reader that in these nerves we have at last found the nerves of nutrition, which have always very properly been bowed out of the front door by the theoretical men and just as often been called in at the back door by the practical men, but I cannot on this occasion follow up this theme farther.

I regret that in the absence of the requisite laboratory facilities I have been unable to perform certain physiological experiments bear-

ing on the questions discussed. I should especially like to repeat the experiments on the chorda of the tongue of dogs anæsthetized by water pressure on the brain by Kemp's method, so that no drugs would have touched the peripheral nerves. I would then try the action of atropia on the nerves of the tongue and see if the formation of the œdema could be thereby prevented, as is the secretion by its action on the nerves of the submaxillary gland. If that should be the case we would have further proof of the correctness of our theory.

I need hardly say in conclusion that I am well aware of the conflict between the views here presented and the current teaching in textbooks, as well as the results reached by many eminent investigators. I am glad, however, to find my conclusions so largely in accord with the opinions of Kölliker and of Cohnheim.

In justice to myself, I wish to say that I am still open to conviction regarding the existence of nerves going to the gland cells. But regarding the formation of lymph and the regulation of its flow, it seems to me that the histological and physiological conditions for this function must exist, and be about the same, all over the body.

CONCLUSIONS.

1. The endings of the motor nerves in striped muscle remain on the outside of the sarcolemma. Aside from the surfaces of contact of muscle and nerve fibre, the end fibres are covered down to their tips with the sheath of Schwann and are provided with nuclei. The precise condition of things at the places of contact of muscle and nerve is an unsolved problem of histology.

2. The ivy-like or festooned arrangement of motor nerves in the frog's muscle has been misinterpreted. Properly interpreted it demonstrates that the nerve fibres that are to influence the muscle fibre are not naked and that they need not be end fibres. It shows that mere contact between muscle fibre and nerve fibre is all that is necessary.

3. The sheath of Henle in the frog and in the smaller muscle fibres of the snake is open, thus permitting escape of the cerebrospinal fluid.

4. In other animals Henle's sheath extends over the end fibres of the

motor nerve and the cells lining it envelop the end fibrils. I find that the so-called "Sohlensubstanz" of Kühne is derived from the cells of Henle's sheath.

5. The terminal nerves in smooth muscle form a network entwining the bundles of muscle fibres. I consider it improbable that each plain muscle fibre has a special terminal nerve fibril.

6. In muscular tissue fine non-medullated nerves, probably belonging to the centrifugal, vasomotor system, proceed from the fasciculi of motor nerves. These nerves can be traced directly to a network of nerves surrounding the capillaries. From this network fine, nucleated, nerve fibres pass to the walls of the capillaries, with which they are very closely united.

7. The nerves supplying the capillaries connect also with sensory nerves and with nerves surrounding the larger blood-vessels, both arteries and veins.

8. The branches of the chorda tympani in the submaxillary gland do not pass to the gland cells, but they terminate on the capillaries.

9. In muscular and glandular tissues—and perhaps throughout the body—there is a vast peripheral nervous plexus belonging to the capillary blood-vessels. These nerves of the capillaries, which may perhaps be regarded as nutritive nerves, regulate the production and transudation of lymph, and are concerned in the mechanism of glandular secretion. They may be called into activity both by peripheral influences and by impulses received from the central nervous system and the sympathetic ganglia. They may influence, through their connections with the vasomotor nerves on the arteries and veins, the blood supply to a part.

THE INFLUENCE OF BILE ON METABOLISM.*

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The influence of the bile on metabolism has been studied heretofore by observing the changes which have occurred when the bile was excluded from the intestinal tract. This paper deals with the results obtained by the administration of bile by the mouth. The incentive to this work came from my friend Dr. Franz Pfaff, to whose unvarying interest and encouragement this paper is chiefly due. In an article by Pfaff and Balch,¹ entitled "An Experimental Investigation of Some of the Conditions Influencing the Secretion and Composition of Human Bile," it was shown that human bile, ox bile and bile salts, when given to a patient with complete biliary fistula, had a marked cholagogic action. Their experiment extended over a period of ninety-seven days, and represents the most complete study of the human biliary secretion which has yet been made. During this investigation it was observed that while the patient was taking bile in one form or another the appetite improved, the bowels moved without medication, and the stools diminished in bulk, but increased in consistency and color.

The article above mentioned closes with the following paragraph: "For the present we will only say that bile may be useful in those cases where so-called cholagogues are now prescribed, as well as in certain cases of constipation, and possibly in cases where we wish to increase the absorption of fat."

The subject² of my experiment was a married woman, fifty-four

* A report of work done under the provisions of the second Dalton Scholarship at the Massachusetts General Hospital for the years 1898-1899.

¹ *The Journal of Experimental Medicine*, 1897, ii, p. 49.

² The patient was in the services of Dr. H. H. A. Beach and Dr. Maurice H. Richardson at the Massachusetts General Hospital, and the operation was performed by Dr. S. J. Mixer. To all these gentlemen I am greatly indebted for the privilege of conducting these investigations.

years of age, who entered the Massachusetts General Hospital September 26, 1898. Her family and personal history were good and she was the mother of twelve healthy children. During the last three years she had suffered from attacks of gall-stones, and on this account came to the hospital for operation. This was performed on September 29. The patient's condition became so critical while upon the operating-table that the gall-bladder alone was emptied of stones and a biliary fistula made, the duct being left untouched.

After the operation the discharge of bile from the wound was constant, the stools remained colorless, and repeated tests failed to show the presence in them of bile acids. It was during the convalescence between December 20 and 31 that the following investigations were made.

The experiment was divided into three periods, similar in all respects save that in the middle period the patient received thirty grammes of dried ox bile. The beginning and the end of the experiment, as well as the different periods, were marked off by the patient's taking the charcoal mixture recommended by F. Müller.³ In each instance the change in the period was definitely shown by the dark color of the stools. The bowels showed no tendency at any time to constipation or diarrhœa. During these twelve days the patient passed about two-thirds of the twenty-four hours in bed, and for the remainder of the day was up and about her room.

All of the food which the patient ate was prepared and weighed in the laboratory. The diet consisted essentially of bread and butter, thin cream, eggs, sugar and beef. The percentage of nitrogen and of fat was determined in each article of food, double analyses being made daily in the case of the cream.

The urine was collected daily and the amount and specific gravity noted. The nitrogen in 5 cc. from the twenty-four hours' amount was determined by the Kjeldahl method, and the urea with Squibb's apparatus, control analyses being made in both cases. The stools for each period, with the wash-water made use of in cleaning the utensils, were made slightly acid with a few drops of concentrated sulphuric

³ Untersuchungen über Icterus. *Zeitschr. f. klin. Medicin*, 1887, xii, p. 45.

acid, and were then evaporated over the water-bath, special care being taken to stir them thoroughly and frequently. The stools did not become solid while over the water-bath, but on removal from the same assumed a firm consistency, though not sufficiently so to allow of reduction to a powder. They were then weighed, and the total percentage of fat determined by double analyses.

The fat was first extracted with ether alone. The residue was then stirred in a porcelain dish with an 8 per cent alcoholic solution of hydrochloric acid and the mixture slowly evaporated to dryness. This residue was then ground up with sand, placed in a fat-extraction thimble and extracted again. It was found simpler to extract the fat in two stages owing to the large amount of fat present in the stools.

The bile was collected at six-hour intervals and the amount, specific gravity and the percentage of solids in the twenty-four hours' amount determined. The fistula caused much annoyance, as it did not admit of the satisfactory use of any cannula. The house officer, Dr. Le-Compte, tried many devices, but it was impossible to prevent leakage. On this account that portion of the bile which was not collected in the flask worn by the patient was caught in the dressings, which were arranged for this purpose, and was subsequently extracted from them with water. This watery extract was then filtered, and the filtrate evaporated to constant weight. As the percentage of solids in the bile each day had been calculated, the amount of bile absorbed in the dressings was easily estimated. That the method was sufficiently accurate is shown by a comparison of the total amount of bile in the first and third periods. In these the amounts of bile are seen to be nearly the same, in spite of the fact that in the first period there was no leakage from the cannula, while in the third period only half the total quantity was collected through the cannula, the remainder being caught in the dressings.

Bile was given to the patient in the second period in the form of bile pills; each pill representing 0.25 gramme dried ox bile. To render these less liable to change in the stomach they were coated with salol—4 grammes to the 100 pills. Of these the patient took 30 daily, a total of 120 in four days, or 30 grammes ox bile and about 5 grammes salol.

Three months after the conclusion of this experiment the patient was operated upon again by Dr. Mixer. The common duct was found to be occluded with gall-stones of soft consistency. These were removed through an incision in the duct, and its complete patency into the duodenum once more established. The fistula ceased to discharge and the patient recovered. The patient then underwent another metabolism experiment of three days' duration, for the determination of her digestion under normal conditions of bile-secretion.

The following tables give the results of these two experiments and require no explanation.

Table I gives the ingesta of the patient during the first period, before bile was given.

Table II gives the ingesta of the patient during the second period, during which bile was taken by the mouth.

Table III gives the ingesta again without bile medication.

Table IV gives the total quantity of bile and bile-solids collected during the three different periods.

Table V gives the excreta of the three periods.

Table VI gives the ingesta in the experiment after the operation by means of which free communication through the bile-duct between the liver and duodenum was once more established.

Table VII gives the excreta in the experiment after the operation.

TABLE I.—INGESTA. 1ST PERIOD. BEFORE BILE MEDICATION.

Wgt. 65.2 kilog. Dec. 20-23, 1898.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread.....	592	8.0	2.7	335
Milk.....	2930	16.2	295.9	143
Sugar....	290			290
Oatmeal	24	0.5		16
Tapioca.....	26			22
Eggs	346	7.0	42.0	
Ham	50	2.3	4.6	
Butter.....	158		136.7	
Cod Fish.....	53	2.8	0.8	
Roast Beef.....	94	3.1	8.9	
Apple Sauce.....	654	0.3		157
	—	—	—	—
		40.2	491.6	963

ANALYSES OF INGESTA TAKEN DURING FIRST PERIOD.

Bread 1.5229 g. contained 0.0207 g. Nitrogen or 1.36 %
 1.9333 g. " 0.0260 g. " " 1.35 Average 1.355 %
 Fat reckoned at 0.46 %. Carbohydrates reckoned at 56.58 %

Milk 2930 cc. in four days.

		Fat.		Nitrogen.
Dec. 20....	910 cc.	11.144 %	11.084 %	0.5264 %
Dec. 21....	490 cc.	8.340		0.5572
Dec. 22....	800 cc.	11.000	10.854	0.5656
Dec. 23....	740 cc.	9.104	9.116	0.5572

Milk Sugar was reckoned at 4.88 %

Oat Meal 2.4686 g. contained 0.0504 g. Nitrogen or 2.04 %
 2.3086 " 0.0466 " " 2.01 Av. 2.025 %
 Carbohydrates reckoned at 64.73 %. König's Tables.

Tapioca Nitrogen neglected. Carbohydrates reckoned at 84.83 %, König.
 3.8813 g. contained 0.0014 g. Nitrogen or 0.036 %
 4.1760 " 0.0015 " " 0.037 Av. 0.0365 %

Eggs Nitrogen reckoned at 2.01 %. König.
 Fat " " 12.11 "

Ham 14.672 g. contained 1.3434 g. Fat or 9.15 %
 Nitrogen reckoned at 4.62 %. See 2d Period.

Butter..... 3.6446 g. contained 3.1900 g. Fat or 87.53 %
 2.8756 " 2.4540 " 85.37 Av. 86.45 %

Cod Fish..... 6.8507 g. contained 0.1072 g. Fat or 1.56 %
 12.2573 " 0.1750 " 1.43 Av. 1.49 %
 2.4827 " 0.1305 Nitrogen or 5.25
 2.6775 " 0.1394 " " 5.20 Av. 5.23 %

Roast Beef..... 38.0 g. contained 3.5796 g. Fat or 9.4 %
 Nitrogen reckoned at 3.35 %

Apple Sauce.... 654 g. made up of 83 g. sugar and 571 g. apples. Carbohydrates
 in latter reckoned at 13.03 %. König. Nitrogen 0.058 %. König.

In addition to the above articles of food, one orange and a small amount of lettuce or celery and a cup of tea and coffee were given daily; also about 75 cc. of sherry in each of the three periods.

TABLE II.—INGESTA. 2D PERIOD. DURING BILE MEDICATION. DEC. 24–27, 1898.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread.....	472	6.4	2.1	267
Milk.....	3575	19.3	355.8	174
Sugar	341			341
Oatmeal	68	1.4		44
Tapioca	31			26
Eggs	560	11.3	67.8	
Ham	39	2.6	1.6	
Butter	114		98.6	
Cod Fish	43	2.2	0.6	
Apple Sauce.....	404	0.2		97
Hamburg Steak...	180	6.0	10.4	
Bile	30	0.5		
		49.9	536.9	949

ANALYSES OF INGESTA TAKEN DURING 2D PERIOD.

For analyses of bread, oatmeal, tapioca, eggs, butter, cod fish, and apple sauce, see 1st Period.

			Fat.		Nitrogen.
Milk	Dec. 24	1090 cc.	10.020 %	9.922 %	
	Dec. 25	865	9.518	9.528	0.5488
	Dec. 26	790	11.166	11.216	0.5166
	Dec. 27	830	9.080	9.324	0.5320

3575 cc.

Milk Sugar reckoned at 4.88 %.

Ham 13.323 g. contained 0.5334 g. Fat or 4.0 %
3.662 g. " 0.1693 g. Nitrogen or 4.62 %

Hamburg Steak. 43 g. taken for analysis; dried to 12.3934 g. for analysis. 5.0414 g. contained 1.0682 g. Fat or 21.69 % or 6.25 % of undried steak.
3.8780 contained 0.7096 g. Fat or 18.30 % or 5.27 % of undried steak.

Average % of Fat = 5.76 %.

1.5856 contained 0.1847 g. or 11.65 % Nitrogen or 3.36 % of undried steak.

Bile009 g. Nitrogen in 2 pills. In 120 or 30 g., 0.54 g.

TABLE III.—INGESTA. 3D PERIOD. AFTER BILE MEDICATION. Dec. 28-31.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread	346	4.7	1.6	196
Milk	2240	11.4	224.9	109
Sugar	463			463
Oatmeal	27	0.5		17
Eggs	504	10.1	61.0	
Butter	96		83.0	
Hamburg Steak . . .	81	2.7	4.7	
Steak	254	9.0	25.1	
		38.4	400.3	785

ANALYSES OF INGESTA TAKEN DURING 3D PERIOD.

For analyses of bread, oatmeal, tapioca, eggs, butter and Hamburg steak, see 1st and 2d Periods.

Milk.	Amount.	Fat.		Nitrogen.
Dec. 28	460 cc.	9.064 %	8.960 %	0.5320 %
Dec. 29 . . .	520	9.764	9.752	
Dec. 30	630	11.496	11.536	0.5348
Dec. 31	630	9.890	9.856	0.4564

2240 cc.

Steak.

Dec. 29 88 g. 26 g. contained 1.9621 g. Fat or 7.55 %
Dec. 30 80 g. 33 " 5.1658 " 15.65
Dec. 31 86 g. 3.6753 " 0.7792 " 21.20 equiv. to
6.37 % undried steak.

3.0106 " 0.7362 " 24.45 " "

7.34 % undried steak. Average 6.86 %

1.7232 contained 0.2015 Nitrogen or 11.69 %

equiv. to 3.51 % " "

1.4864 " 0.1756 " " 11.81 %

equiv. to 3.55 % " "

Average 3.53 %

TABLE IV.—EXCRETION OF BILE IN THE THREE PERIODS.

Period.	Cannula.	Extracted from Dressings.	Total.	% Solids.	Total Solids.
I. Before Bile Medication,	2250	0	2250	1.37	30.83
II. During Bile Medication,	1429	1079*	2508	1.70	42.64
III. After Bile Medication,	1173	751*	1924	1.42	27.32

*The dressings were extracted several times with water until all trace of bile was removed. The extracts were then filtered and the filtrates evaporated and dried to constant weight. In Period II the dried bile-solids thus obtained were 18.3819 g. But the per cent of solids of the bile obtained in this period by the cannula was 1.70%. . . $1.70 : 100 :: 18.3819 : x =$ equivalent amount of bile obtained by extraction, i. e., 1079 cc. In Period III the dried bile solids obtained from the dressings amounted to 10.6772 g.—equivalent to 751 cc.

TABLE V.—EXCRETA. 1ST, 2D, AND 3D PERIODS.

URINE.					
		Amount.	Sp. Gr.	Urea.	Nitrogen.
I. Before Bile Medication,	Dec. 21	510	1029	14.6	8.1
	Dec. 22	400	1024	10.1	5.6
	Dec. 23	460	1024	12.7	7.2
	Dec. 24	630	1018	13.5 50.9	7.6 28.5
		2000			
II. During Bile Medication,	Dec. 25	1224	1009	14.6	7.3
	Dec. 26	840	1016	18.2	9.4
	Dec. 27	700	1017	13.7	7.7
	Dec. 28	670	1022	14.9 61.4	9.3 33.7
		3434			
III. After Bile Medication,	Dec. 29	670	1021	15.2	10.1
	Dec. 30	650	1018	17.2	9.5
	Dec. 31	650	1018	17.2	9.5
	Jan. 1	270	1020	7.2 56.8	3.8 32.9
		2240			
STOOLS.					
		Amount.	Nitrogen.		Fat.
I. Before Bile Medication,		423	1.79-7.6%		73.3-310%
II. During Bile Medication,		298	1.19-3.5		71.8-214
III. After Bile Medication,		290	1.51-4.4		78.1-226

ANALYSES OF THE EXCRETA OF THE THREE PERIODS.

1st Period.	Stools.	423 g. contained	310 g. Fat and 7.6 g. Nitrogen.
		5.0970 g. contained	3.7034 g. Fat or 72.66%
	3.3647	"	2.4884 " 73.96 Average 73.31%
	2.5962	"	0.0456 Nitrogen or 1.76
	3.2571	"	0.0588 " 1.81 " 1.79

2d Period.	Stools.	298 g. contained 214 g. Fat and 3.5 g. Nitrogen.			
3.7352 g.	contained	2.7154 g.	Fat or	72.69%	
5.0952	"	3.6166	"	70.98	Average 71.84%
3.4500	"	0.0396	Nitrogen or	1.15	
3.1200	"	0.0382	"	1.23	" 1.19
3d Period.	Stools.	290 g. contained 226 g. Fat and 4.4 g. Nitrogen.			
4.6836 g.	contained	3.5994 g.	Fat or	76.85%	
4.2176	"	3.3432	"	79.27	Average 78.06%
2.3090	"	0.0344	Nitrogen or	1.49	
2.4366	"	0.0372	"	1.53	" 1.51

SUMMARY OF THE FIRST EXPERIMENT.

	Fat		Nitrogen		Bile Solids.	Balance Excreta, urine and stool.	Nitrogen.
	Ingesta	Excreta faeces.	Ingesta	Excreta faeces.			
I. Before Bile							
Medication.	491.6	310 or 63.1 %	39.9	7.6 or 19 %	30.83	36.1	+ 3.9
II. During Bile							
Medication.	537.1	214 or 39.8	49.7	3.5 or 7	43.64	37.2	+ 12.6
III. After Bile							
Medication.	400.3	226 or 56.5	38.4	4.4 or 11.5	27.32	37.3	+ 1.1

The digestion of fat was respectively 23.3 per cent and 16.7 per cent better in the bile period than in the 1st and 3d periods, in which no bile was given.

The digestion of nitrogenous food was respectively 12 per cent and 4.5 per cent better in the bile period than in the other periods.

The bile-solids were increased in the bile period 38 per cent and 60 per cent respectively above the amounts in the 1st and 3d periods. The excess of bile-solids in this period was equivalent to nearly one-half the amount of bile given during this time.

TABLE VI.—INGESTA IN THE EXPERIMENT AFTER THE OPERATION.

Weight 65.1 kilog. May 20-24, 1899.				
Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread.....	423	5.8	1.9	239
Milk.....	2470	12.1	194.6	120
Sugar.....	363			363
Oatmeal.....	58	1.2		38
Tapioca.....	18			15
Eggs.....	707	14.2	85.6	
Butter.....	73		61.7	
Beef Steak.....	147	4.9	16.9	
Chicken.....	188	10.7	8.6	
Potato.....	229	0.5		44
		49.4	369.3	819

ANALYSES OF INGESTA TAKEN DURING THE EXPERIMENT AFTER THE OPERATION.

For bread, oatmeal, tapioca, and eggs, see analyses 1st Period. Experiment 1.

		FAT.		NITROGEN.	
Milk					
May 2....	810 cc.	8.25 %	8.38 %	0.4662 %	
May 3....	900	7.43	7.35	0.5166	
May 4....	760	7.96	8.03	0.4914	
Butter.....	2.3100 g. contained	1.9524 g.	Fat or	84.5 %	
	1.9780	"	1.6714	"	84.4 Average 84.45
Beef Steak.....	5.6178 g.	"	0.6450 g.	"	11.48
Nitrogen reckoned at 3.36%—amount obtained at a subsequent analysis.					
Chicken	3.2096 g. contained	0.1616 g.	Fat or	5.03 %	
	1.8238	"	0.1050	"	5.75 Average 5.39 %
	3.1382	"	0.1770	Nitrogen or	5.63
	2.9700	"	0.1711	"	5.75 " 5.69
84 g. of first portion of chicken given.					
Chicken	2d Portion 104 g.	Nitrogen reckoned at 5.69 % as in 1st Portion.			
	2.4930 g. contained	0.0864 g.	Fat or	3.46 %	
	3.8758	"	0.1386	"	3.57 Av. 3.52 %
Potato	37.8 g.	"	0.2630 g.	Carbohydrates or	19.0 %
	37.6	"	0.2616	"	" 19.1 " 19.05
	8.0	"	0.0172	Nitrogen or	0.215
	8.0	"	0.0168	"	" 0.21

TABLE VII.—EXCRETA DURING THE EXPERIMENT AFTER THE OPERATION.

URINE.				
	Amount.	Sp. Gr.	Urea.	Nitrogen.
May 3.....	400	1032	8.8	5.2
May 4.....	1070	1017	23.2	11.8
May 5.....	975	1017	19.4	8.4
	2445		51.4	25.4
STOOLS.				
	Amount.	Nitrogen.	Fat.	
	145	5.9 g. or 4.1 %	54.8 g. or 37.8 %	

ANALYSES OF EXCRETA DURING THE EXPERIMENT AFTER THE OPERATION.

STOOLS.				
145 g. contained 54.8 g. Fat and 5.9 g. Nitrogen.				
4.9832	"	1.9020 g.	Fat or	38.1 % Average
5.8335	"	2.1826	"	37.4 37.8 %
1.4572	"	0.0596	Nitrogen or	4.1 Average
2.3256	"	0.0952	"	4.1 4.1 %

SUMMARY OF THE EXPERIMENT AFTER THE OPERATION.

FAT.		NITROGEN.	
Ingesta.	Excreta.	Ingesta.	Excreta.
369.3 g.	54.8 g. or 14.8 %	49.4 g.	5.9 g. or 11.9 %

SUMMARY OF THE TWO EXPERIMENTS.

	Per cent. of ingested fat lost in stools.	Per cent. of ingested nitrogen lost in stools.	Bile solids.	Urine per day.
I. Before Bile Medication,	63.1%	19.0%	30.83 g.	500 cc.
II. During Bile Medication,	39.8	7.0	43.64 g.	859 cc.
III. After Bile Medication,	56.5	11.5	27.32 g.	560 cc.
After operation,	14.8	11.9		815 cc.

Four other experiments were also performed—one on a human being and three on dogs with biliary fistulæ. Since these failed to be satisfactory in all particulars, only a brief resumé of this part of the work will be given.

Experiment 1 was performed upon a healthy woman, October 4-16, 1897. She was given in the second period of four days 20 grammes of dried ox bile. Notwithstanding the fact that the amount of fat in the food reached nearly 200 grammes a day, it was so well assimilated that it left no appreciable chance for improvement in its absorption during the bile period.

Experiment 2 was performed on a dog February 5-25, 1898. The common duct was ligated in two places and severed. The gall-bladder was then opened and a glass cannula, extending for 4 cm. beyond the abdominal wall, inserted. The dog recovered quickly from the operation and at the end of two weeks the wound was so well healed that he was ready for use. The diet consisted of dog-bread ground up and mixed with lard. The animal was kept in a cage with double bottom and fed twice a day. The experiment was divided into four periods, in the third of which the dog was given 12 grammes ox bile.

PER CENT. IN STOOLS OF INGESTA.

		Fat.		Nitrogen.
Feb. 5-10	I Without muzzle			
	No Bile	51.82 per cent.	24.31 per cent.	
Feb. 10-15	II With muzzle			
	No Bile	45.87 per cent.	19.70 per cent.	
Feb. 15-20	III With muzzle			
	Bile	59.95 per cent.	34.23 per cent.	
Feb. 20-25	IV With muzzle			
	No Bile	33.15 per cent.	16.67 per cent.	

The second period showed a loss of fat in the stools of 46 per cent as contrasted with 52 per cent in the first period. Such figures, however, are not conclusive when one is dealing with large amounts of fat.

The third and fourth periods are not of value because the dog had diarrhœa, due either to the bile pills or to the fat in the food. This not only interfered with the absorption of the fat, but prevented accurate separation of the stools.

Experiment 3. The dog was prepared in the same way as in the preceding experiments. He wore a muzzle throughout the experiment and for the three and one-half days preceding. The experiment was of 15 days' duration (June 30 to July 15, 1898), and divided into periods of five days each. In the second of these, the dog received 17.5 grammes of the dried ox bile.

Yet though the amount of fat in the diet was lessened from 80 grammes to 40 grammes a day, diarrhœa appeared as before aggravatingly near the end of the bile period. The results were, therefore, inconclusive.

PER CENT. IN STOOLS OF INGESTA.

	Fat.	Nitrogen.
I	41.9 per cent.	14.9 per cent.
II	45.3 "	12.4 "
III	63.0 "	22.1 "

Experiment 4. Dog. Common duct ligated and biliary fistula made June 18, 1898. In this instance the three periods were of two days' duration. In the middle period the dog took 10 grammes dried ox bile.

PER CENT. IN STOOLS OF INGESTA.

	Fat.	Nitrogen.
July 19-21,	46.1 per cent.	16.8 per cent.
July 21-23,	34.2 "	11.8 "
July 23-25,	74.5 "	31.8 "

But here again diarrhœa occurred toward the close of the experiment, vitiating the results.

The literature on this subject up to 1897 was given in detail by Pfaff and Balch ⁴ in their paper. Albu ⁵ has since brought the matter up to date. It will be only necessary therefore for me to state my conclusions.

⁴ Op. cit.

⁵ Zur Physiologie und Pathologie der Gallensecretion, *Berl. klin. Wochenschr.*, 1900, p. 866.

CONCLUSIONS.

1. Bile increases the digestion of fat when given by the mouth in pill form. The percentage of fat lost in the stools of our patient with a complete biliary fistula was 63 per cent in the first period and 57 per cent in the third. This closely corresponds to the results that Müller obtained in human beings and dogs with complete obstruction of the common duct. Under bile medication the stools contained 23 per cent less fat than in the first period, and 17 per cent less than in the third. This represents an actual diminution of the amount of fat lost in the stools. Looking at the result in another way, it may be said that the average digestion of fat in the periods without bile was 40 per cent; in the periods with bile, 60 per cent, *i. e.*, bile increased the digestion of fat relatively by 50 per cent.

2. The digestion of nitrogenous food is improved by the use of bile pills when the amount of fat in the stools is large. Instead of an average of 15 per cent being lost in the fæces, but 7 per cent escaped digestion during the four days the patient took bile. The reason for this, perhaps, lies in the better digestion of fat at this time, in consequence of which the proteid elements of the food were more thoroughly exposed to the digestive juices.

3. Ox bile is a cholagogue. The amount of bile-solids secreted in the bile period was 47 per cent greater than in the periods before and after. This confirms the work of Pfaff and Balch, here in Boston, on a human being, and that of Stadelmann⁶ and his pupils, in Germany, on dogs.

4. The effect of the bile on the bowels in this case was not remarkable, although they moved more satisfactorily during the bile period. In my experiments with dogs I usually obtained diarrhœa when giving bile. I do not feel sure, however, that this should be attributed wholly to the medication, for the diarrhœas as a rule appeared six or more days after the beginning of the experiment and the animals were then in poor condition. Dr. Pfaff, who has had more experience with the administration of bile than I, tells me that he has found its action variable in patients. In some cases it is a laxative; in others, in

⁶ *Zeitschr. f. Biol.*, 1897, n. F., xvi, p. 1.

which there is diarrhœa, due apparently to large amounts of fat in the food, it has the opposite effect.

5. As to the general effect of bile on body metabolism, it was observed that the urea and nitrogen were excreted in greater amount in the bile period than in either of the others. No definite conclusions can be drawn from this fact, because more nitrogen was ingested during these four days; moreover, it must be borne in mind that in these results the salol may have been a factor.

6. The amount of urine was increased by more than 50 per cent in the bile period. It is interesting to note that the amount was about the same during this bile period as in the second experiment when the bile was again taking its natural course. Von Noorden⁷ has recorded a similar increase in the amount of urine following the removal of the obstruction in acute catarrhal jaundice. The salol coating of the bile pills, which amounted to one and a quarter grammes a day, is not sufficient to account for this effect. This is evident from the work of Kumagawa,⁸ who gave two grammes of sodium salicylate daily to a dog of 25 kilos without essentially changing the amount of urine secreted. On the other hand, in taking the 30 pills daily the patient drank several extra glasses of water, and in the second experiment her general condition was naturally better than at any other time.

⁷ *Pathologie des Stoffwechsels*, p. 281, Berlin, 1893.

⁸ *Virchow's Archiv*, 1888, cxiii, p. 134.

THE RELATION OF DIABETES MELLITUS TO LESIONS OF THE PANCREAS. HYALINE DEGENERATION OF THE ISLANDS OF LANGERHANS.

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PLATE XXXIII.

Embedded in the substance of the pancreas are the peculiar bodies described by P. Langerhans and usually designated islands of Langerhans. They are composed of polygonal cells arranged in irregular columns, between which are wide tortuous anastomosing capillaries. The cells are of epithelial type and have the same origin as those of the ducts and secreting acini with which, at an early period of development, the cell columns are in continuity. The lumen of the ducts does not penetrate among the cells of the island, which is, therefore, not concerned in the elaboration of the pancreatic juice. These bodies resemble in architecture other ductless structures, such as the parathyroid bodies, the carotid and coccygeal glands, and less closely the suprarenal capsules, the pituitary body and the thyroid gland.

The intimate relation of columns of epithelial cells to a rich capillary network has suggested that they furnish some substance to the blood—the hypothetical internal secretion of the pancreas. Abundant experimental research, inaugurated by von Mering and Minkowski, having shown that the pancreas exerts some very important influence on carbohydrate metabolism, several writers—Laguesse, Schäfer, Diamare—have suggested that the islands of Langerhans perform this function. The only experimental evidence in support of this suggestion is furnished by Ssobolew, who, in a brief preliminary communication, states that after feeding animals with carbohydrates in considerable quantity the cells of the islands become

more granular than usual. He finds that the chronic interstitial pancreatitis, which in dogs follows ligation of the pancreatic ducts, spares the islands of Langerhans; this fact, he thinks, explains the absence of glycosuria.

In the preceding number of this JOURNAL¹ I have described alterations undergone by the islands of Langerhans in various forms of chronic interstitial pancreatitis, and have discussed the relation of these lesions to the disease of carbohydrate metabolism, diabetes mellitus.

Two forms of chronic inflammation of the gland are distinguishable: (1) interlobular pancreatitis, characterized by proliferation of fibrous tissue between the lobules, which are invaded from the periphery, and (2) interacinar pancreatitis, where the new-formed fibrous tissue is more diffusely distributed within the lobules and between individual acini. With the first type the islands of Langerhans are implicated only when the sclerotic process has reached a very advanced grade. To this variety belongs the chronic inflammation which follows occlusion of the pancreatic duct; though the secreting tissue of the gland is in very great part destroyed and replaced by dense fibrous tissue, the interacinar islands are not affected and persist for a long time as isolated cellular structures almost completely unchanged, though surrounded by scar-like tissue. They suffer only when the process is far advanced. With the interacinar type of inflammation, on the other hand, the islands are affected as are the other elements of the gland, and coarse strands of fibrous stroma following the capillary vessels separate the columns of atrophied cells.

Of eleven instances of chronic pancreatitis of the interlobular type, in only one was diabetes present. Here the inflammation which followed obstruction of the pancreatic duct had reached a very advanced

¹ Opie. On the relation of chronic interstitial pancreatitis to the islands of Langerhans and to diabetes mellitus. *Journal of Experimental Medicine*, 1901, v, p. 397. References to the authors cited are here given. See also *Journal of the Boston Society of the Medical Sciences*, June, 1900, iv, p. 251. W. Schulze (*Arch. f. mikr. Anat.*, August 31, 1900, lvi, p. 491) has recently shown that the islands of Langerhans are not implicated in the chronic interstitial inflammation which follows obstruction of the pancreatic duct in guinea pigs.

grade and the islands of Langerhans, isolated in the dense stroma, had undergone alterations. The accompanying glycosuria had been of only slight severity and had disappeared when the patient was upon a diet poor in carbohydrates. In two of three instances of interacinar pancreatitis diabetes mellitus was present. In the third case the lesion was slight and the organ weighed 170 grammes. Though the number of cases is small they indicate that where diabetes accompanies a lesion of the pancreas the islands of Langerhans are implicated in the disease.

In the same report I described a case of diabetes mellitus in which the pancreas was the seat of a very remarkable lesion. In sections from all parts of the organ were small areas in which the parenchymatous cells were replaced by hyaline material located immediately outside the walls of the capillaries. These areas, which were most abundant in the tail of the gland, frequently corresponded in size to islands of Langerhans, but were often much larger. Self-digestion of the gland in many places prevented the satisfactory study of the lesion, but even where the tissue was well preserved islands of Langerhans were not recognizable. Although the change began apparently in these structures, it had extended beyond their limits.

I have recently had the opportunity of studying the specimens from a case of diabetes in which the causal relation of a lesion of the islands of Langerhans to the disease is more clearly demonstrable than in those previously described. These bodies are the seat of a degenerative change which has left unaltered the secreting parenchyma of the gland.

Synopsis of Clinical History.—Female, negro, aged 54 years. The patient was admitted to the Johns Hopkins Hospital in the service of Dr. Osler, complaining of cough. Her family and personal history are unimportant. Her present illness began about eleven months before her admission, when, she states, she had a severe cold which became steadily worse. The cough has been accompanied by profuse expectoration. She has lost much weight. Several months after the onset of cough her urine increased greatly in quantity, so that for a time she was compelled to get up almost every hour during the night to void it. The urine was pale in color. At this time she experienced great hunger

and thirst and ate and drank enormously. These symptoms lasted during part of the spring and summer and disappeared some months before her admission to the hospital. She had recently voided the usual amount of urine and there was no excessive hunger or thirst.

When admitted the patient was thin but moderately well nourished. The mucous membranes were pale. The percussion note over the front of the chest and in the axillary region was hyperresonant; over the upper part of the back on the right side the note was dull, while elsewhere over the back there was flat tympany. The breath-sounds over the first and second interspaces on the right side in front were intensely tubular, while on the left side above the fourth interspace they had an amphoric quality and were almost cavernous. There was modified tubular breathing over the left back, amphoric over the upper part of the scapula. Numerous fine and coarse moist râles were heard throughout both lungs. A friction rub was audible in the lower left axilla. The sputum was abundant, yellowish green, muco-purulent in character, and contained numerous tubercle bacilli. The hæmoglobin was 62 per cent. The stools were of normal color and contained no fat.

The patient gradually became weaker. Cough was almost constant and large quantities of muco-purulent material were expectorated. The temperature was irregular, and until the day preceding death ranged between 98.8° and 102° F. Death occurred on the seventh day after admission and was not preceded by a period of coma.

The specific gravity of the urine varied between 1025 and 1035. It contained sugar in abundance; neither albumin nor casts were found. On the fourth day after admission 880 cc. were collected, the specific gravity was 1028 and 4 per cent of sugar was present. On the following day the amount was 1200 cc., the specific gravity 1035 and the quantity of sugar 5.4 per cent.

Autopsy.—Performed 51 hours after death. The body is that of a sparsely nourished woman; the arms and legs are very thin and the abdomen is retracted. Subcutaneous fat is present in small amount.

The heart-muscle is pale, and upon the intima of the mitral valve are opaque, yellow patches of fatty degeneration. Within the coronary arteries near their orifices are a few slightly raised yellow sclerotic patches.

The left lung is very voluminous and is almost universally bound by fibrous adhesions to the chest wall. Immediately below the pleura, occupying the upper part of the upper lobe, is a large, irregular cavity, the walls of which are covered by yellowish necrotic material. The re-

mainder of the lobe is consolidated, dull yellowish red, mottled with areas of caseation and riddled with small cavities. The upper part of the lower lobe is very thickly studded with groups of confluent partly caseous tubercles. The right lung is also voluminous and bound at the apex to the chest wall by fibrous adhesions. Upon the pleura are sparsely scattered gray tubercles. At the apex below the pleura is a cavity, which in size and appearance resembles that of the left lung. The remainder of the lobe is in great part consolidated by numerous caseous tubercles; the tissue is tough in consistence and contains much fibrous tissue. Caseous tubercles are abundant in the middle and lower lobes. Upon the mucosa of the larynx near the posterior extremities of the vocal cords and upon the surface of the epiglottis are a few very superficial ulcers, the largest about 3 mm. in diameter.

The liver is pale and its lobulation is marked by gray-yellow and red mottling. Upon the cut surface are seen rather conspicuous yellowish points of minute size. The spleen is not enlarged.

The kidneys are of large size and weigh together 400 grammes. The surface, after removal of the capsule, is smooth and pale. The cortex, which has an average thickness of 5.5 mm., is of a grayish-red color. The left kidney has two separate pelves, from which arise two ureters opening by separate orifices into the bladder.

The mucous membrane of the stomach is normal in appearance. In the lower part of the ileum are a few scattered superficial ulcers about 0.5 cm. in diameter with irregular, slightly raised edges. On the peritoneal surface opposite one of them are several gray nodules which are just visible. A few similar ulcers are present in the large intestine.

The pancreas weighs 80 grammes and measures 23 x 5 x 1 cm. It can be readily dissected from the surrounding tissues. It is soft in consistence, and on section has a gray-yellow color.

The intima of the aorta, though fairly smooth, is studded with irregular, slightly raised plaques. The arteries at the base of the brain are normal in appearance. No lesion of the brain is found; the floor of the fourth ventricle presents nothing unusual. In the lower part of the right lateral lobe of the thyroid gland is a round firm nodule about 1 cm. in diameter. Its cut surface has a bright yellow color and is mottled with red and translucent gray.

Microscopic Examination.—In sections of the pancreas prepared for histological study is found no generalized increase of the interstitial tissue, but here and there, particularly in the tail of the organ, the

fibrous stroma shows some proliferation, and there are occasional irregular strands of tissue between the acini, though these are almost universally separated by delicate septa. This scanty, new-formed interstitial tissue, where it occurs, is poor in cells; about some of the medium-sized interlobular blood-vessels are small accumulations of scattered lymphoid cells, together with an occasional plasma cell. Mononuclear cells with eosinophile granulations are not infrequently seen in the interlobular and interacinar tissue. In the head and the body of the organ are small areas of post-mortem self-digestion, where nuclei no longer stain and the tissue takes with hæmatoxylin a diffuse blue tint. The glandular tissue elsewhere is well preserved. In many acini, centro-acinar cells are numerous. The ducts are not dilated and appear to be normal. There are no alterations of the veins or arteries.

The islands of Langerhans are the seat of a very remarkable change (Plate XXXIII). In varying amount within almost every island is a homogeneous material which stains with eosin. Only rarely is found an unaltered island. Those which are least changed contain a few scattered masses of hyaline material, of which the smallest are irregularly polygonal in shape and correspond in size to the cells of the island. The larger particles are rounded. This hyaline substance at times lies in the midst of groups of cells, but is usually in contact with the walls of the capillaries penetrating the island, or next the peripheral fibrous tissue, and is therefore usually between the remaining cells and the capillary walls. Increasing in amount, it replaces the cells and, where it is abundant, the cells which still persist are small and contain small nuclei, staining deeply with hæmatoxylin. They do not appear compressed or distorted.

Where hyaline material is abundant it forms conspicuous masses in contact with capillaries, the endothelium of which is well preserved. It does not form a uniform zone about them, but it occurs as scattered groups of irregular, rounded, often globular masses (Plate XXXIII, Fig. 2). The cells of the island have been in large part replaced, and between the hyaline particles is seen only an occasional compressed, fusiform or irregular nucleus.

The hyaline substance may occupy almost the entire area of the island, and besides a few endothelial cells are found only small scattered groups or rows of atrophic epithelial cells. The island is represented by a sharply circumscribed, hyaline structure, composed of particles of homogeneous material, giving the impression of broken, twisted columns, between which are the capillary walls. The nuclei of the capillary endothelium persist after destruction of the epithelial cells, but finally disappear. The lumen of the capillary remains patent and red blood-corpuscles are seen between the hyaline masses, although the endothelium no longer contains nuclei. The hyaline metamorphosis is limited strictly to the islands of Langerhans, the glandular acini remaining intact.

Lesions similar to those of the pancreas are not present in other organs. In the heart-wall are a few small areas where the interstitial tissue is increased, and in places the muscle-cells are fragmented, but other changes are not found. Sections from the lung show the histological pictures of conglomerate tubercles and of gelatinous and caseous pneumonia. The blood-vessels of the liver, spleen and kidney are apparently unaltered and there is no formation of hyaline material in these organs. Miliary tubercles are present in the liver, whose cells contain much fat.

The nodule observed at autopsy in the thyroid gland consists of altered parenchyma circumscribed by a thin circular capsule of fibrous tissue. The alveoli composing it vary greatly in size, some being very small and containing no colloid material, while others are large and irregular and distended with this material. The interstitial tissue of the nodule has an almost uniform hyaline appearance, when stained with eosin, and contains only very few scattered nuclei. The epithelium of the dilated alveoli is often much flattened. In places it is broken and the colloid within is continuous with the homogeneous substance replacing the interstitial tissue. Treated with Van Gieson's stain, the contents of the greater number of alveoli stain only with picric acid and assume a bright yellow color; but occasionally this material is colored by fuchsin and becomes deep orange red. The hyaline material immediately outside the alveoli and continuous, where the epithelial lining is broken, with that within, takes the same yellow stain and is doubtless extravasated colloid. Where it infiltrates the interstitial tissue midway between adjacent alveoli, it contains those constituents of the

fibrous stroma which have an affinity for fuchsin and, therefore, assumes a reddish color. The thyroid alveoli outside the nodule are far less irregular in size, and the stroma does not show the same uniform hyaline transformation, but here and there extravasation of colloid material has occurred into the interstitial tissue.

The peculiar transformation affecting the islands of Langerhans in this case belongs to the varied and ill-defined group of degenerative processes whose common characteristic is the formation of a homogeneous or hyaline material which stains with acid dyes, such as eosin and picric acid, but does not give the reactions of amyloid substance, though it resists the action of a variety of chemical substances, as strong acids and alkalies. These characteristics have been used by von Recklinghausen² to group together products of cell degeneration occurring in widely different tissues and doubtless representing a variety of essentially different processes, which even yet have received no satisfactory classification.

Under the heading of colloid transformation von Recklinghausen has included amyloid, hyaline and mucous degenerations—processes whose common character is the formation of a substance insoluble in the tissue juices. He recognized that the chemical and physical peculiarities ascribed to hyaline material do not serve to identify it as a true chemical compound, but he has grouped together as the products of related processes the hyaline substances which are formed in many different situations. These include the hyaline formed in the choroid of the eye, in certain tumors of the connective tissues and of the lymphatic apparatus; the homogeneous substances which are formed by certain glandular cells as products of secretion or of degeneration, namely, the contents of thyroid alveoli, hyaline renal casts, etc.; material formed on the surface and in the superficial layer of mucous membranes as the chief constituent of diphtheritic membranes; the so-called waxy or pseudo-waxy material formed by acute degeneration of smooth and striated muscle-fibres; the hyaline thrombi formed in the blood-vessels and in the heart.

Klebs³ has attempted to classify more precisely these heterogeneous

² Handbuch der allgemeinen Pathologie des Kreislaufs und der Ernährung, p. 404. Stuttgart, 1883.

³ Die allgemeine Pathologie, Theil ii, p. 100. Jena, 1889.

processes and to define more clearly certain long-accepted terms which have been applied to them. He reserves the name "colloid" for those albuminous substances which resemble the colloid of the thyroid gland and, like it, are elaborated by secreting cells, though not necessarily preformed within them. As "hyaline" he designates the firm, refractive and homogeneous albuminous material which does not give the reactions of amyloid and is formed in connective tissue and in other derivatives of the mesoderm. The hyaline material formed in and outside the cardiovascular system from constituents of the blood, the hyaline thrombi and hyaline exudates he distinguishes from the tissue hyaline produced by transformation of connective tissue.

By means of staining reactions, P. Ernst⁴ attempts to demonstrate that the various substances grouped together by von Recklinghausen as hyaline are not chemically identical. Hyaline material from different sources, when treated with Van Gieson's mixture, exhibits in its affinity for acid fuchsin and picric acid differences which he attributes to peculiarities of chemical composition. That which is derived from epithelial cells stains orange yellow with the mixture of the two dyes, being colored by the picric acid and only tinted by fuchsin. Examples of this form of hyaline are the colloid of the thyroid gland and many renal casts. The second variety is formed in connective tissue or from coagulated material derived from the blood and stains intensely with fuchsin. Such hyaline occurs in the interstitial tissue of the thyroid gland, in certain tumors, as the hyaline remnants of the corpora lutea of the ovary, and as hyaline glomeruli in the kidney.

Lubarsch⁵ regards the staining reactions of Ernst as an uncertain means for the identification of different hyaline substances and points out discrepancies between the criteria proposed by Klebs and by Ernst. Hyaline thrombi, which, according to Ernst, should stain red are usually yellow and only rarely stain deeply with fuchsin. The renal casts, which stain by Weigert's method for the demonstration of fibrin, stain orange yellow, though they are, as Ernst himself thinks, hyaline fibrin and, therefore, of parablasic origin. The colloid contents of the thyroid alveoli (as in the nodule described above), occasionally stain deep red, although this substance is undoubtedly of epithelial origin. Although Lubarsch discards the classification of Ernst, he admits that the method employed indicates in many cases the origin of hyaline

⁴ Virchow's *Archiv*, 1892, cxxx, p. 377.

⁵ *Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie. Herausgegeben von Lubarsch u. Ostertag*, 1895, ii, p. 200.

material. Its advantage, he believes, lies in the fact that by it we can recognize whether hyaline contains a constituent derived from connective tissue. Normal fibrous tissue is stained intensely red by acid fuchsin and the substances which determine its affinity for the dye are present in the degenerate tissue. Pure hyaline, whatever its origin, Lubarsch suggests, always stains in the same way, while differences in staining reaction are dependent upon the admixture of other substances.

Hyaline material formed by a variety of processes from different tissues and exudates doubtless differs in chemical composition. That which is produced by coagulative necrosis from the protoplasm of parenchymatous cells and by the so-called pseudo-waxy degeneration of muscle-fibres is not identical with the hyaline of thrombi and of hæmatogenous exudates. The hyaline transformation of connective tissue in various situations, in certain tumors, in the walls of capillaries, in altered renal glomeruli, represents a longer continued process and is more closely related to amyloid degeneration. Since we have at present no means of determining the nature of these substances, their source and their methods of production can alone be used as a basis of classification. Following Lubarsch, we may distinguish hyaline of epithelial and of conjunctival origin, and again we may recognize that, like the colloid of the thyroid gland, formed outside of cells presumably by a process of secretion, and that formed by transformation of the cell protoplasm.

The material which in the case herewith described partly or completely replaces the islands of Langerhans, was tested with a variety of agents which have been used in the study of hyaline substances. It stains deeply with acid dyes, like eosin and picric acid, but shows little affinity for nuclear stains, as, for example, hæmatoxylin and methylene blue. With basic fuchsin and water blue, by the method which Unna⁶ has used for the demonstration of basiphilic hyaline, it is stained only by the acid dye. The tissue available for study after discovery of the lesion had been hardened in Zenker's fluid. The methods employed by Pianese⁷ in studying the hyaline degeneration occurring in cancer cells require in great part special methods of fixation and were not used.

⁶ *Monatshefte für prakt. Dermatol.*, 1894, xix, p. 663.

⁷ *Ziegler's Beiträge z. path. Anat.*, Suppl. Heft, 1896, p. 684.

The reactions of amyloid were not obtained with iodine, nor with gentian violet, methyl violet nor iodine green. The material did not stain by Weigert's method for the demonstration of fibrin.

Of much interest, in view of the study of Ernst, is the behavior of the substance toward picric acid and acid fuchsin. It stains with picric acid, but shows no marked affinity for acid fuchsin. The result varies slightly when varying proportions of the two substances are used. With a mixture of 3 parts of concentrated aqueous solution of acid fuchsin and 150 parts of concentrated aqueous solution of picric acid (Van Gieson-Ernst) the hyaline is stained yellow tinted with red. Using the two stains, as employed by Unna, a pure yellow results; the minute strands of fibrous tissue which penetrate the island stain deep red, while the hyaline material in contact with them is yellow.

This material, therefore, conducts itself toward Van Gieson's stain as does, according to Ernst, hyaline of epithelial origin, and in its staining reaction resembles the colloid material which is present in the greater number of thyroid alveoli and which in the thyroid nodule described has found its way into the interstitial tissue. Accepting the interpretation of the reaction suggested by Lubarsch, it does not contain the constituent of fibrous tissue upon which depends an affinity for acid fuchsin. It may be here noted that in the thyroid nodule, where extravasated colloid has infiltrated the fibrous tissue between adjacent alveoli, it has assumed a reddish color.

The staining reactions of this homogeneous substance present in the islands of Langerhans, interpreted with reserve, indicate its epithelial origin. Finding it in irregular masses in contact with the cells, I was at first inclined to believe that it was formed by a process resembling secretion. It was pointed out to me by Dr. Welch, who examined my specimens, that transitions occurred between the cells and the hyaline masses. In slightly altered islands one finds small masses of material which resemble the hyaline substance, but, like the cell protoplasm, have a granular aspect. They correspond in size to the adjacent cells of the island but contain no nuclei.

By the use of certain stains, phosphomolybdic acid hæmatoxylin

by the method of Ribbert for white fibrous tissue or aniline blue, as employed by Mallory,⁸ for the demonstration of white fibres and reticulum, the hyaline material acquires a deep blue color and becomes very conspicuous. In the islands are not infrequently found slightly enlarged cells which, though still containing nuclei, exhibit a reaction similar to that of the hyaline material. The cell protoplasm, though granular, has assumed a diffuse blue color.

The degenerative process first manifests itself by an increase in the size of the cell and an alteration of its protoplasm. With the death of the cell its nucleus disappears and the protoplasm which stains with acid dyes remains for a time granular, but subsequently becomes homogeneous. The small particles of hyaline fuse with one another and form larger masses which lie in contact with the fibrous septa of the island. After complete transformation of the cells the island is represented by a hyaline mass penetrated by the remains of altered capillaries.

In the preceding number of this JOURNAL⁹ I described, with an illustrative drawing, a case of diabetes in which hyaline material was present in circumscribed areas throughout the pancreas. The staining reactions of this substance resembled those observed in the present case. Amyloid reactions were not obtained, nor did it stain by Weigert's method for the study of fibrin. With the various modifications of Van Gieson's mixture, it showed little affinity for acid fuchsin. With the methods of Ribbert and of Mallory previously mentioned, it assumed a deep blue color. The deposition of calcium salts within it gave evidence that the process was of long standing. Unlike that of the present case, this substance was deposited in fairly compact tortuous columns; the areas of hyaline transformation frequently corresponded in size to islands of Langerhans, but in many cases had evidently extended beyond their limits; transitions between the epithelial cells and the hyaline substance were not noted. Owing to the poor preservation of the tissue, it was not possible to study the early stages of the condition. It is nevertheless not improbable that the lesions in the two cases are of similar nature.

⁸ *Journal of Experimental Medicine*, 1900, v, p. 15.

⁹ *Op. cit.*, p. 419.

Varied experiments performed upon the pancreas of animals have conclusively shown that this organ is essential to normal carbohydrate metabolism, and abundant clinical and pathological observation has demonstrated that the conclusions based upon these experiments are applicable to man. In my previous study I have briefly summarized the experimental and clinical evidence which establishes the causal relationship of certain destructive lesions of the pancreas to diabetes mellitus.

Impaired pancreatic function is doubtless not the only cause of diabetes, and in many instances of this disease no alteration of the organ has been demonstrable. On the other hand, diabetes is present in only a limited number of the cases where pancreatic disease has existed. It was the object of my preceding article to show that, where diabetes is caused by a lesion of the pancreas, the lesion is of such a character as to destroy or injure the islands of Langerhans, and that where, though the organ is diseased, diabetes is absent, the interacinar islands are relatively unaffected. In the cases (Nos. XIV and XV) of chronic interacinar pancreatitis, accompanied by diabetes, sclerosis of the organ was so slight that it was definitely recognized only by the microscope, while in instances (Nos. XI and XII) where the lesion followed obstruction of the pancreatic duct, diabetes was absent, though the organ was in great part converted into fibrous or fatty stroma. In the former cases the lesion affected the islands of Langerhans, while in the latter they were spared, though the surrounding secreting tissue was destroyed. In no instance were the islands destroyed, while the secreting structures remained intact.

Structures so embedded as are these islands in the substance of the gland cannot by any means now at our disposal be subjected to experimental alterations without injury to the surrounding alveoli. In the pancreas which has been described in this article, a lesion of obscure etiology has destroyed the cells of the islands of Langerhans, while those of the secreting acini, as well as those of other organs, are unaffected. The most successful experiment could not more accurately have selected these bodies. The association of diabetes mellitus with this lesion affords, I believe, convincing proof of the inferences drawn from the preceding series of cases.

Destruction of the pancreas in animals and in man is accompanied by diabetes; in the present case destruction of the islands of Langerhans has been accompanied by this disease. Since diabetes is absent when, as the result of duct obstruction, the secreting portion of the gland undergoes great alteration, though the islands are spared, the conclusion is justified that it is those structures which influence carbohydrate metabolism. What has been learned concerning the relation of the pancreas to diabetes is the relation of the islands of Langerhans to this disease.

DESCRIPTION OF PLATE XXXIII.

Fig. 1. Drawing made with low magnification (Leitz Oc. 3; Obj. 3) showing hyaline transformation of islands of Langerhans.

Fig. 2. Drawing made with a higher magnification (Leitz Oc. 3; Obj. 6) showing an island whose cells are partly transformed into hyaline material.

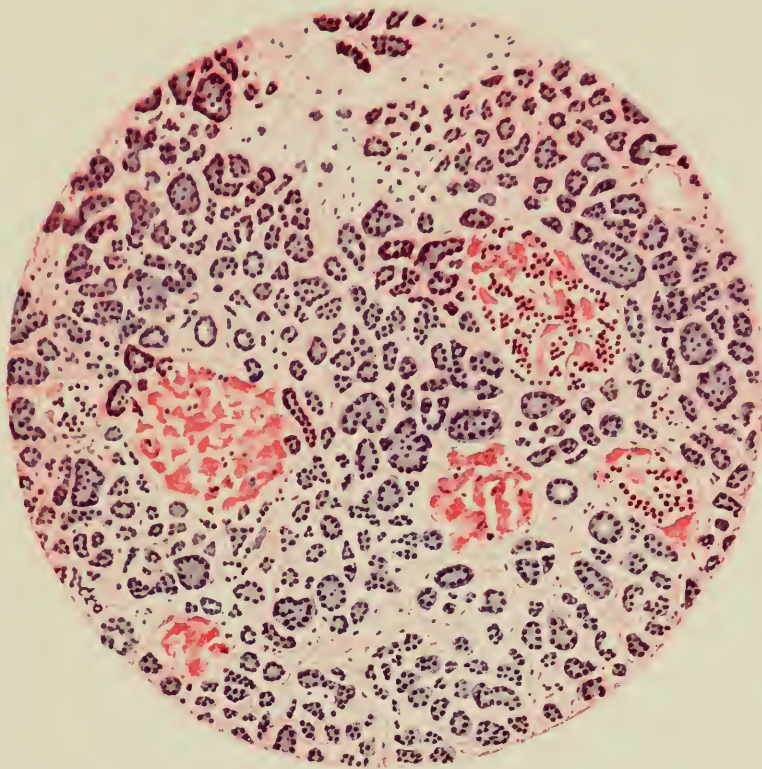


FIG. 1.

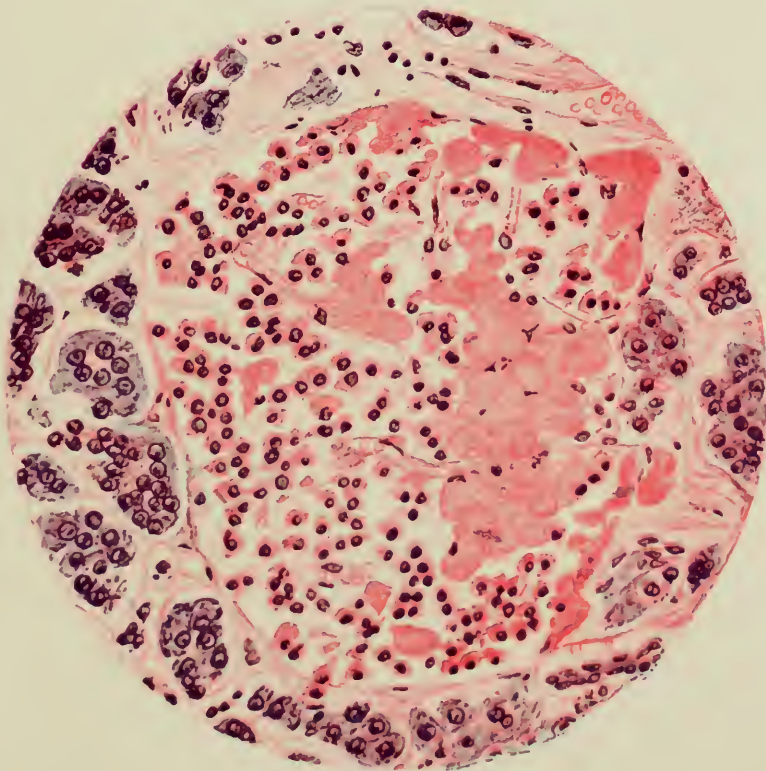
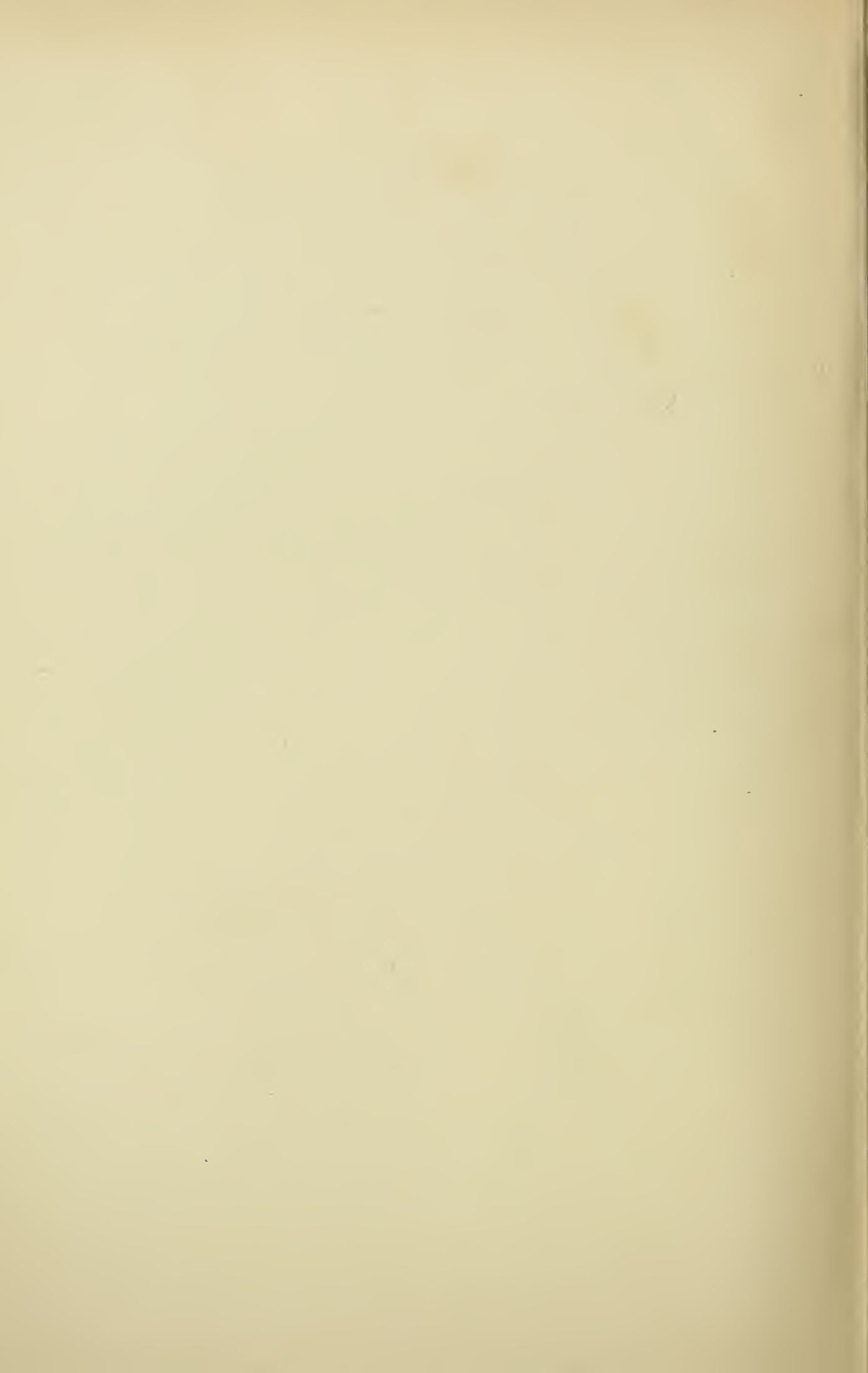


FIG. 2.



ALLOXURIC EXCRETION IN A CASE OF LEUCOPENIA.

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INTRODUCTORY.

It is now nearly half a century since it was first pointed out by H. Ranke¹ that the excretion of uric acid is increased in certain cases of leucocythæmia. Since then nearly fifty independent workers have repeated the investigations, and with few exceptions have corroborated Ranke's observation. Amongst those who obtained a contrary result may be mentioned Mosler² and Jacubasch,³ but it must be remarked that in the cases investigated by these observers other factors presented themselves which might be held accountable for the absence of increase. Thus in Mosler's case there was very marked debility with a correspondingly low nitrogen excretion, whilst in Jacubasch's patient the existence of œdema and diarrhœa may account for the exceptional result.

In most of these observations, however, the method employed to estimate the uric acid was that of Heintz, which has since been shown to be far from correct. Since the introduction of the more exact methods of Hopkins, Salkowski and Ludwig the number of cases examined has not been great, and of these only a few are suitable for comparison with the physiological excretion, the composition of the diet having been left out of account in the majority of cases.

¹ Beobachtungen und Versuche über die Ausscheidung der Harnsäure beim Menschen. München, 1858.

² Virchow's *Archiv*, 1866, xxxvii, p. 45.

³ Virchow's *Archiv*, 1868, xliii, p. 217.

Where both these sources of inaccuracy have been eliminated, the general result of the investigations has been to show that an increase in the number of leucocytes does not necessarily go hand in hand with a rise of endogenous alloxuric bodies in the urine, but that only in cases of spleno-medullary leucocythæmia is a distinct increase present.⁴ On the other hand, the lymphatic form of the disease seems to show no increase, whilst leucocytoses, whether physiological⁵ or produced by the administration of drugs,⁶ are devoid of any constant effect. It would therefore seem that Horbaczewski's theory, which connects an increase of alloxur bodies with a corresponding leucocytosis, does not hold good for all forms of leucocytic increase.⁷

Investigations to decide the question of a parallelism between the number of leucocytes and the alloxuric excretion have usually been carried out on cases of leucocythæmia, whereas very few observations have been recorded in cases in which the number of leucocytes was diminished.

In the following case, where a distinct leucopenia was present, we estimated the amount of alloxuric nitrogen excreted in the urine each day during two periods of eight days each. In doing this consideration was taken of the valuable work of Burian and Schur,⁸ who pointed out that, before any estimate could be formed of the amount of the bodies excreted, a careful regulation of the diet was necessary in order to be certain that endogenous purins only were excreted, for it is in them that any increase or decrease is of importance, since they alone arise from the metabolic processes in the tissues.

CLINICAL HISTORY.

Mrs. B., aged 37, a widow, was admitted to the London Hospital in the beginning of June, 1900, complaining of "general weakness." Her ill-

⁴ Milroy and Malcolm, *Journal of Physiology*, 1898, xxiii, p. 235, and 1899, xxv, p. 109.

⁵ Sivén, *Skandinav. Arch. f. Physiologie*, 1900, xi, p. 123, and Kühnau and Weiss, *Zeitschr. f. klin. Med.*, 1897, xxxii, p. 482.

⁶ von Noorden and Zuntz, *DuBois-Reymond's Archiv, Physiol. Abth.*, 1894, p. 203.

⁷ For details see Schreiber, *Ueber die Harnsäure unter physiol. u. pathol. Bedingungen*. Stuttgart, 1899.

⁸ *Arch. f. d. gesamte Physiologie*, 1900, lxxx, p. 241.

ness had begun about twelve months before admission immediately after she left Gibraltar, where she had been living for about a year. Previous to this she had always enjoyed good health. There was no evidence of her having suffered from either syphilis or malaria. She was a poorly nourished, fair woman (weight 7 stones), pale and of somewhat yellowish complexion.

There was no jaundice, cyanosis nor dropsy. There were a few purpuric spots round the ankles, but no other evidence of hæmorrhage either in the skin or retina.

The spleen was enormously enlarged, extending down beyond the umbilicus into the right iliac fossa. The edge was rounded; the surface somewhat irregular and slightly tender. The liver was also enlarged, the lower border being three inches below the costal margin in the mammary line. Its surface was smooth and firm. There was no glandular enlargement and no ascites. No abnormality was detected in the thoracic organs, nervous system or urine.

The blood contained $4\frac{1}{2}$ million red corpuscles, 65 per cent of hæmoglobin, and 1500 to 3000 white corpuscles. The lymphocytes and polynuclears were practically equal in number. No eosinophiles and no abnormal elements were seen in several stained films.

The temperature during her stay in the hospital was persistently irregular, usually running up to 103° F. or 104° F. in the evening. The pyrexia exhibited a tendency to occur in periods of 10 or 12 days, separated by 2 or 3 days of almost normal temperature. She had occasional bleedings from the nose and a tendency to diarrhœa, but ate well and maintained the same weight throughout.

At first the case was regarded as one of splenic anæmia and was treated on that assumption without improvement. The fact, however, that her illness had begun shortly after leaving Gibraltar aroused the suspicion that she might be suffering from Malta fever. The reaction of her serum to *Micrococcus melitensis* (Bruce) was accordingly tested by Dr. Bulloch. The serum was found to agglutinate the cocci in the proportion of one part of serum to 30 of culture, and in accordance with this the conclusion was arrived at that the case was one of Malta fever. The patient remained in hospital until the end of 1900 without exhibiting any marked change in her condition, though the leucocytes had increased to about 3000 per cubic millimetre under treatment with nuclein. From a clinical

point of view the case was remarkable as exhibiting an absence of the joint pains usually present in Malta fever and the presence of a much greater degree of splenic enlargement and of leucopenia than are usually found in that disease.

METHODS.

During both periods the patient was confined to bed, and was kept on a diet consisting of eggs, milk, bread and farinaceous foods, but containing no flesh nor other purin-yielding ingredients. The exact amount of food consumed was ascertained, and the urine was collected for each day. The faeces were neglected, since no appreciable amount of purins is lost with them.

The total nitrogen was estimated by Kjeldahl's method, and the nitrogen of the total alloxuric bodies by that of Camerer. Parallel estimations were made in each sample during the second, and in most of those of the first period.

RESULTS.

TABLE I.—1ST PERIOD.

Date.	N. of Food*.	Urine Amount.	Total N.	Alloxuric N.
July 2	16 gm.	812 cc.	9.630 gm.	0.1867 gm.
3	14	1315	6.996	0.1330
4	14.5	1475	8.152	0.1652
5	17	1350	11.1321	0.2295
7	16	1965	10.779	0.1224
8	16	1750	11.2000	0.1224
9	15	1940	14.550	0.2211
10	15	1965	12.3402	0.1670

* Calculated from König's tables.

During this experiment the patient received approximately about 15 grammes of nitrogen in the diet, but this amount varied somewhat and was accompanied by corresponding swings in the urinary nitrogen. It would seem as if a considerable nitrogen retention was taking place in the tissues, but the patient had several attacks of diarrhoea during the investigation, whereby some nitrogen may have been lost. The total alloxuric nitrogen averaged about .17 gm. in the 24 hours, but was on two occasions as high as .22 gm., and on another as low as .122 gm. Such variations in the endogenous alloxuric excretion have been denied by Burian and Schur to exist physiologically, but, as these observers point out, they may occur in disease. During the

period of examination, also, it is probable that, on several occasions, a certain amount of urine was lost with the fæces. The average, however—namely, .17 grm.—falls within the limits of the physiological excretion, this varying between .122 grm. and .20 grm.

TABLE II.—2D PERIOD.

Date.	N. of food.	Urine Amount.	Total N.	Alloxuric N.	Leucocytes.	S. P*.
Sept. 4	17.2 grm.	1140 cc.	13.23 grm.	0.2042 grm.	2184	1 : 1
5	18	1695	15.36	0.2084		
6	18	1560	12.036	0.2085	1560	3 : 2
7	18	1465	10.814	0.1871	
8	17.5	1205	10.567	1872	2 : 3
9	18.5	1365	12.558	0.2064	3000	5 : 4
10	19	1100	13.530	0.2002		1 : 1
11	19	1570	14.750	0.2103		11 : 10

* S. signifies simple nucleated leucocytes; P. signifies polymorphonuclear leucocytes.

The investigation recorded in Table II was carried out about two months after the previous one, and during it the patient's general condition was much better, there being no diarrhœa. The urine was collected with greater care, and only on one occasion (namely, on Sept. 8) was there any admixture with fæces.

The diet was free from alloxur bodies and contained rather more nitrogen than on the previous occasion. The total alloxuric nitrogen showed only slight variations, its average being 0.20 gr. in the 24 hours. This is considerably more than during the previous period, which may be accounted for by the improved condition of the patient and the more careful investigation of the case. It still falls within the normal for alloxuric nitrogen however, although at the highest limit of this.

CONSIDERATION OF RESULTS.

The average number of leucocytes per cubic millimetre during the two periods was 2500, sometimes reaching as high as 3000 or falling to 1800. Regarding their nature, the most important point is the relative increase of lymphocytes, or, more correctly stated, the decrease in the total leucocytes affected the polymorphonuclear cells to a greater extent than the lymphocytes. This great diminution in the granular cells might conceivably be due to one of two causes:

1. To an increased destruction, the production remaining normal.
2. To a diminished production, the rate of destruction remaining normal.

If the former of these conditions were present, the amount of disintegration products of the leucocytes excreted by the urine—*i. e.*, of alloxuric bodies and of phosphorus—would be increased. If the latter obtains a diminution in these would be expected. In the case before us, however, the excretion falls within the normal, though at the highest limit of this; and if one considers that the patient weighed only 45 kg., and had probably a low endogenous factor, then it may be assumed that a slight increase was present, and therefore that the leucopenia was occasioned by active destruction of the polynuclears in the enlarged spleen, and that the red bone-marrow had not been able to make good the loss. It would seem as if in this regard the only difference between this case and one of spleno-medullary leucocythæmia was the bone-marrow activity. In leucocythæmia this organ is more active than normal, thus making good the increased destruction by the spleen and even overcompensating it, whereas in leucopenia it has not compensated for the splenic destruction.⁹

Another clue to the amount of leucolysis occurring in the body is, as Milroy and Malcolm¹⁰ have pointed out, the amount of phosphorus excreted. In ascertaining this, however, the ingested phosphorus must be very carefully estimated, which fact introduces serious difficulties when we come to apply the method to patients. On four days following the second period we estimated the total phosphorus excreted with the following results:

TABLE III.

Date.	Urine Amount.	Total P ₂ O ₅ *.
Sept. 18	1100 cc.	2.266 gm.
19	1570	2.386
20	1220	2.170
21	1300	2.418

* Estimated by titration with uranium nitrate solution, using tincture of cochineal as indicator.

⁹ In this connection, however, it should be pointed out that it is conceivable that both formative and destructive organs may act on a high or on a low level. In the former case the number of leucocytes might remain constant, but their life history be of short duration, and the alloxuric excretion consequently increased, whereas, in the latter case, where both organs were depressed in activity, and the life history of the leucocytes of long duration, a normal number of leucocytes would be accompanied by a diminished alloxuric excretion.

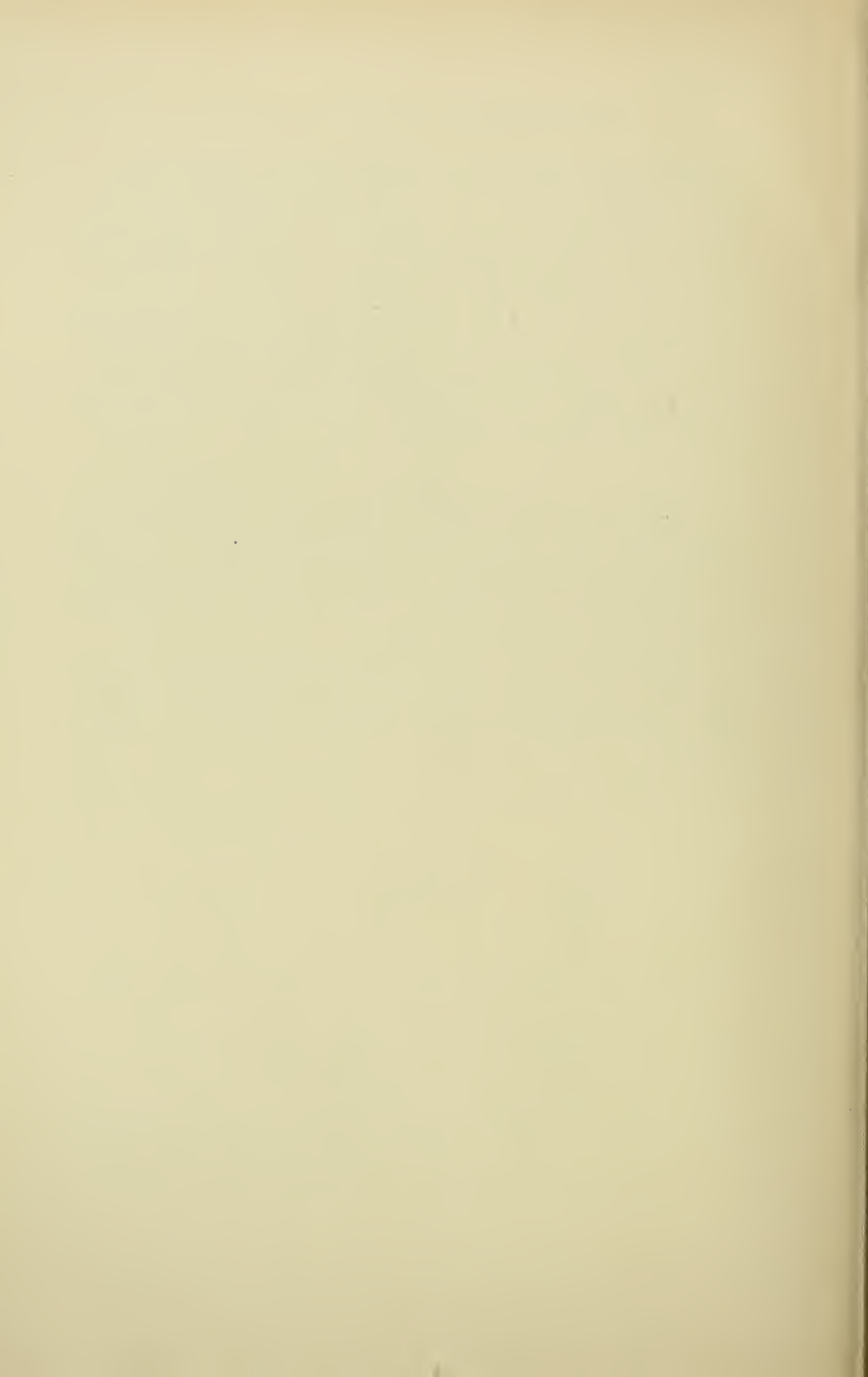
¹⁰ *Journal of Physiology*, 1898, xxiii, p. 217, and *ibid.*, 1899, xxv, p. 105.

The diet was similar to that during the previous examinations, and was of exactly the same amount each day, containing probably a little less phosphorus than an ordinary mixed diet. From this it would appear that, if anything, the excretion of phosphorus was slightly increased, pointing to an increased leucolysis.

A consideration of these different points renders it probable that the leucopenia in this case was due to an inability on the part of the red bone-marrow to make good an increased leucolysis in the spleen. The entire absence of any myelocytes or other abnormal blood-cells would seem to support this opinion.

SUMMARY.

1. In the case investigated, one of Malta fever, the leucocytes were reduced to between 1500 and 3000 per cubic millimetre.
2. Notwithstanding this, the alloxur bodies and the phosphoric acid in the urine, the patient being on an alloxur-free diet, showed no distinct diminution from the normal.
3. The suggestion is made that this result may be due to the leucopenia being brought about by an increased destruction of leucocytes in the spleen rather than to a diminished activity of the bone-marrow.



STUDIES ON THE MORPHOLOGY OF GANGLION CELLS
IN THE RABBIT.

- I. THE NORMAL NERVE CELLS.
II. CHANGES IN THE NERVE CELLS IN RABIES.

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PLATES XXXIV-XXXVIII.

CONTENTS.		PAGE
I.	THE NORMAL NERVE CELLS.....	549
	Introduction.....	549
	Historical review.....	551
	Methods.....	556
	1.—Spinal and medullary stichochromes.....	558
	The nucleus and nuclear contents.....	559
	The cell body.....	561
	2.—Nerve cells of the spinal ganglia.....	567
	3.—Nerve cells of the cerebellum.....	568
	4.—Nerve cells of the olfactory lobe.....	569
	5.—Nerve cells of the cerebral cortex.....	573
	6.—Nerve cells of the basal ganglia.....	578
II.	CHANGES IN THE NERVE CELLS IN RABIES.....	581
	Historical review.....	581
	Material and methods.....	584
	Personal observations.....	585
	Bibliography.....	607
	Description of plates.....	611

I.—THE NORMAL NERVE CELLS.

PLATES XXXIV AND XXXV.

INTRODUCTION.

The purpose of these investigations was the study of the changes which take place in the nerve cell as the result of disease, and especi-

ally the sequential order of these changes from their inception to their termination. With this end in view, the plan was to take up in turn the study of the sequence of nerve-cell changes in a number of experimentally produced diseases; the aim being to determine if possible, by a comparison of the results in the different diseases, either a specific type of cellular changes or sequence of changes for each separate morbid process, or a unity in the effects of different morbid conditions upon the nerve cell, and thus to deduce, if possible, some general laws governing the sequence of morbid changes.

The advantages, in fact the necessity of using animals and experimental methods in such a study, are obvious. Aside from the ability to obtain material at pleasure from any desired stage of a disease, is the advantage of completely eliminating post-mortem changes, which are an almost constant possible source of error in material obtained from human autopsies. The animals used were the rabbit and the guinea-pig.* These have the advantage, on the one hand, of being such common subjects of experimental inoculation that their reactions to the various bacteria and their toxins are comparatively well known, thus rendering unnecessary extensive control series, and on the other hand, of approximating sufficiently in organization to man to warrant some attempt at least at analogical deductions.

At the very outset a complete barrier to accurate pathological work was encountered in the lack of satisfactory data, and especially of accurate drawings of the nerve cells of the normal rabbit and guinea-pig. In studies such as the present, involving investigations of cytological changes, an extremely accurate norm of comparison is essential. Originally intended merely as a necessary preliminary to the pathological work, this study of the normal cell has constantly increased in scope and importance until it now makes a large part of the work. Yet if it may furnish to other investigators in this field of experimental pathology a basis for comparison and eliminate for them

* I am at present engaged in work upon the nerve cells of the normal guinea-pig and upon the changes in guinea-pigs subjected to the tetanus toxin, and to the diphtheria toxin; and also upon the changes in the nerve cells of rabbits inoculated with the poisons of the rattlesnake and of *Heloderma*. See preliminary report before the New York Academy of Medicine in the *Medical Record*, (New York), June 2, 1900.

the necessity of going over the normal, it may be of service. At the same time, throughout the studies in normal cytology, the original purpose has been kept clearly in view. Thus there has been no attempt at a study of all the nerve cells of the normal rabbit and guinea-pig. Only the cells from certain definitely selected regions of the normal central nervous system have been selected for study in order that they might furnish a basis for comparison with cells from the same regions of probably pathological nervous systems in the same animals.

The first part of this paper is devoted to a description of normal nerve cells of the rabbit, and the second part (p. 581) to a consideration of the changes in these cells in rabies.

HISTORICAL REVIEW.

Before presenting the results of our study of the structure of the nerve cell in the normal rabbit, it is desirable to review briefly the present state of our knowledge concerning the internal structure of the nerve cell in general. A full presentation of the existing knowledge upon this subject is contained in Barker's admirable book "The Nervous System and its Constituent Neurones" (31).

So profound an influence had the introduction of Nissl's methods of staining nerve cells, the first of these published in 1885 (1), that it is convenient to divide the history of the development of our knowledge of the internal structure of this cell into the periods before Nissl and after Nissl. In the earlier period the majority of investigators described the internal structure of the nerve cell as fibrillar in character. Between the fibrils was a ground or basement substance, homogeneous or finely granular, about which little was said. Opposed to this early idea of a fibrillar structure of nerve cells was a smaller group of investigators, among them Key and Retzius (2), and Arndt (3), who described the protoplasm of the cell as homogeneous or granular, with larger masses scattered through it; to an arrangement of these granules in rows or threads they attributed the fibrillar appearance noted by other writers.

The principles of Nissl's method are extremely simple. Briefly, they consist in the use of a quick fixative, such as alcohol, and subsequent staining with one of the basic aniline dyes, such as methylene blue. Nissl recognizes in the structure of nerve-cell protoplasm stained by

his method (certain variable pigmented deposits excepted) two distinct substances: 1, a stainable substance, which reacts strongly to basic aniline dyes; and 2, a non-stainable substance, i. e. one which fails to react to basic aniline dyes.

Based upon this reaction and upon variations in arrangement, which the stainable substance presents in different cells, Nissl (4) has formulated a system of classification of nerve cells as follows:

1. Cells which react only as to their nuclei to the basic anilines, the cell body remaining unstained. Of these cells, Nissl recognizes two subgroups:

(a) Cytochromes. These are the cells formerly spoken of as "granules," those of the molecular layer of the cerebellum being an example. The nucleus is no larger than that of the neuroglia cell.

(b) Caryochromes. The cell body is usually free from chromatin and consequently unstained. Rarely a small bit of stainable substance is found to one side of the nucleus. The nucleus resembles that of other nerve cells, and is larger than that of neuroglia cells.

In neither of these cells is there by this method any definitely outlined cell body, and yet that they have very definite cell bodies, and that to at least one other stain these bodies present the same reaction as do those of other nerve cells, has been shown by the method of Golgi.

2. Cells which react both as to their nuclei and as to their cell bodies to the Nissl stain. To these cells Nissl has given the name "somatochromes." He recognizes three subgroups:

(a). Arkyochromes or cells in which the chromatic substance is present in the form of anastomosing chains, making a coarser or finer meshwork; as, for example, in the Purkinje cells of the cerebellum and in the mitral cells of the olfactory lobe. Again subdividing, he describes two types of arkyochrome cells:

(α). Enarkyochromes or cells in which the nodal points of the network are united by fine threads of chromatic substance.

(β). Ampharkyochromes or cells in which the nodal points are united by heavier bands or bridges which take a dense stain.

(b). Stichochromes or cells in which the chromatic substance is arranged in more or less distinctly parallel rows, the direction of the rows usually bearing some relation to the contour of the nucleus and to that of the periphery of the cell body. To this group belong such cells as the large cells of the ventral horn of the cord, the spinal-ganglion cells, some of the cells of the cornu Ammonis, and some of the cells of the cerebral cortex.

(c). Gryochromes or cells in which the chromatic substance is present

in the form of fine granules. Such cells Nissl describes in the corpus striatum.

This classification Nissl at first believed to be of both morphological and physiological significance; i. e. that each morphological type of cell corresponds to a physiological function. While this appears to be true within certain narrow limits, the classification is now accepted mainly in a morphological sense. The importance of this classification in the present instance is that Nissl claims, and is supported therein by many other investigators, that these types are perfectly definite, not only for man but also for lower animals; that a cell of a given type found in a given region in the human nervous system, will be found to occur in precisely the same or analogous region as we pass downward through the animal series. Thus in studying cells from a particular locality in the nervous system of an animal, we should expect to find a correspondence in type to cells found in the same or analogous localities in man and in other animals, the differences being only within the range of variation of that type.

Again recognizing a variation in staining reaction among cells of the same type—a variation quite constantly found, and believed to be explicable by chemical changes dependent upon the metabolic condition of the cell—Nissl has classified cells of the same kind according to their different chromatic reactions. In some cells the chromatic elements are closely packed and the cell consequently takes a dense stain; these he describes as in the pyknomorphous condition. In others, the chromatic elements are more loosely distributed, and these cells, staining less intensely, are said to be in the apyknomorphous condition. A mean condition between these two is designated as parapyknomorphous. A condition in which the entire cell body stains intensely, there being apparently, little or no non-stainable element present, is designated by Nissl as chromophilic. The significance of these cells is not clear. Their occurrence seems to be more common after some fixatives than after others, and they are said to be more frequent in portions of the tissue exposed to the direct action of the reagent. Opinion tends to their interpretation as artefacts.

The physical and the chemical nature of this stainable element of the nerve cell, or more properly, of that element of the cell body which presents such a definite reaction to the Nissl stain, still furnishes a wide field for investigation and discussion. Von Lenhossék (5), Held (6), de Quervain (7) agree that this substance, as seen in sections of hardened material, is, however homogeneous in appearance, really granular in character. Following up this idea, Held and von Lenhossék inves-

tigated the conditions in fresh material. The latter, in spinal-ganglion cells, freshly teased in an indifferent medium, could see, in addition to the nuclear structure, only a finely granular protoplasm, without definite structure or arrangement. Held's investigations, while corroborating those of von Lenhossék, went farther. Fresh nerve cells, examined immediately after removal in a passive medium, showed distinctly outlined nuclei with nucleoli, but in the protoplasm of the cell bodies no formed elements whatever. These remained perfectly clear and homogeneous, or slightly granular, depending largely upon rapidity of technique, being the more clear, the sooner after removal the cells were examined. With methylene blue, in dilute solutions, he obtained a reaction showing granular masses, even in cells so fresh as to show no structure when examined unstained. He considers this due to a fixative action of the methylene blue. By subjecting these fresh cells to the action of the various fixing agents commonly used in the Nissl technique, Held claims to have observed in his previously clear protoplasm the formation of granular masses, which he considers identical with the Nissl bodies as revealed by the usual Nissl procedure. In sections of hardened material, stained blue with erythrosin and methylene blue, he finds the Nissl granules embedded in a homogeneous coagulum-like mass, which stains violet in contra-distinction to the blue of the granules and to the red of the acidophile elements of the cell.

Investigations have been made also by Held, Halliburton (8), Macallum (9), and others upon the chemistry of the Nissl bodies with the following general results: They are soluble in alkaline fluids of all strengths, and insoluble in acids, ether, and alcohol. They respond to tests for phosphorus and, according to Macallum, to tests for iron. As a result of his investigations, both histological and chemical, Held concludes that in the Nissl bodies we are dealing with a phospho-albumin, similar to the nucleo-albumins; that in the living cell this phospho-albumin exists in solution among the other ingredients of the protoplasm; that the action of fixatives is to cause a coagulation or precipitation of the albuminoid substance and in this way to give rise to the Nissl picture. Opposed to this view are the findings reported by Flemming of masses representing Nissl bodies in fresh cells.

As to the structure of that portion of the cell body which does not react to the Nissl stain, three main theories, each founded upon the studies of its originator, and supported with certain minor modifications by what may be termed his "school," exist:

1. That the structure is fibrillar in character. Originating with Remak (10) and Max Schultze (11) long before the introduction of the

Nissl ideas, it has been supported by Ranvier (27), Boll (28), Schwalbe (29), Kronthal (12), Dogiel (13), in a modified form by Flemming (14) and E. Müller (15), and more recently by Bethe (16), Becker (17) and Apáthy (18). That all of these believers in the fibrillar structure of what we may call the ground substance of the nerve cell, are in agreement as to the structure and arrangement of these fibrillæ is far from true. The early supporters of this doctrine looked upon the nerve cell as essentially fibrillar, the fibrils being merely held together by a web of interfibrillar cement. Bethe and Apáthy, among the recent investigators, strongly support the fibrillar theory. Bethe describes minutely the course of the fibrils entering and leaving the cell by its various processes, the fibrils seeming to pass through the cell body, rather than to be an integral part of its organization. Apáthy's ideas of both the intra-cellular and the extra-cellular courses of the fibres are even more complex than are Bethe's. Both as yet need confirmation.

2. Bütschli (30), extending to nerve cells his theories concerning the foam-like structure of protoplasm in general, sees in these cells a honeycomb structure, the whole cell body being made up of a great number of superimposed elementary cells or compartments after the fashion of a honeycomb. He explains the discrepancies in the results of other investigators as largely due to the different pictures presented by these structures, depending upon thickness and upon direction of the plane of section. In some of his most recent writings, Held seems to subscribe, with certain reservations, to the theory of Bütschli. He believes, however, that these honeycomb structures represent artefacts of fixation, rather than structures preexistent in the protoplasm of the living cell.

3. The idea of a distinct reticular structure within the non-stainable substance of Nissl receives the support of Ramon y Cajal (19), Held (20), van Gehuchten (21), Van Gieson (22), and Ewing (23); Held, as already stated, accepting Bütschli's theory as a possible explanation of the reticular appearance. This reticulum traverses with its strands a more fluid element, the cytolymph or cytoplasm. Held describes in very thin sections, stained with erythrosin and methylene blue, a granular appearance of this reticulum, and in the axis-cylinder process an arrangement of these granules in rows, giving the appearance of striations or fibrillæ.

Special attention has been called by Rosin (24), to the micro-chemical differentiation of the constituents of the cell body, the Nissl bodies reacting to the basic dyes, while the ground substance is acidophilic in character.

METHODS.

Material.—For the study of normal cells, six rabbits were used. Those were selected which had been for some time under observation, none having been previously used for experimental purposes, or having shown any sign of disease. All were perfectly healthy normal rabbits. Rabbits of medium weight were chosen without reference to sex. The internal organs were normal.

Methods of Killing.—Rabbits Nos. 1 and 2 were killed by chloroform in an air-tight jar of about ten litres, so that beyond the amount originally in the jar, no air was admitted with the chloroform; there may therefore have been some asphyxiation. These rabbits were unconscious at the end of three minutes, when dissection was begun.

Rabbit No. 3 was killed by chloroform in the open air, dying in about five minutes.

Rabbit No. 4, killed by ether in a jar partially air-tight, died in about six minutes.

Rabbit No. 5, killed by cutting the throat, bled to death almost instantly.

Rabbit No. 6 was killed instantly by a blow on the back of the neck.

From the standpoint of convenience, the method of placing the animal in an air-tight jar with sufficient chloroform to kill it, is the most satisfactory. The other methods served as controls, it seeming easily within the range of possibility, that certain cells, as, for example, those of the olfactory lobe might present modifications in their finer structure due to the action of such irritant substances as ether, chloroform, or carbonic-acid gas. It was with this possibility especially in view that the methods adopted for rabbits 5 and 6 were selected.

Comparative studies upon the effects on the finer structures of the nerve cell of these different methods of killing showed that no specific effects—at least none demonstrable by the Nissl technique—were produced by the different modes of death used. Cells from rabbits killed by the different methods and then subjected to the same technical procedures, presented an extremely uniform appearance.

Fixation and Hardening.—In all cases the brain, cord and spinal ganglia were removed as quickly and as carefully as possible, cut transversely into thin slices by a sharp razor wet with normal saline solution, and transferred immediately to the fixative.

Rabbit No. 1.—Alternate sections of the brain and cord were put in:

1.—Lang's fluid.....	{	Mercuric chloride	5.
		Sodium chloride.....	6.
		Acetic acid	5.
		Water.....	100.

2.—Van Gehuchten's fluid	{	Acid acetic gl.....	10.
		Chloroform.....	30.
		Alcohol abs.....	60.

In each of these fluids the material was allowed to remain 12 hours. That subjected to the Lang fixation was then passed through graded alcohols 40%, 60%, 80%, to absolute. To alcohols 80% and absolute, tincture of iodine was added until decolorization ceased. Van Gehuchten's fluid was followed by absolute alcohol, changed frequently until all trace of acid reaction had disappeared.

Rabbit No. 2.—The central nervous system was fixed in situ, by injection through the ascending aorta of normal salt solution at 100° F., followed by a 10% aqueous solution of formalin at the same temperature. Brain, cord, and ganglia were then removed and placed in formalin 10%, followed by graded alcohols.

Rabbit No. 3.—Fixation of alternate slices in: 1. Absolute alcohol. 2. Formalin 5% for 13 hours, followed by graded alcohols.

Rabbit No. 4.—Van Gehuchten's fluid.

Rabbit No. 5.—Fixation of alternate slices in: 1. Van Gehuchten's fluid. 2. Formalin 5%.

Rabbit No. 6.—Fixation of alternate slices in: 1. Absolute alcohol. 2. Formalin 10%.

Embedding.—For most of the work celloidin was used. It allows of sections quite thin enough for comparative purposes. Sections 6 μ in thickness are easily obtained and may be taken in series so as to include the whole of a particular cell. With care in embedding, sections 4 μ thick may be made, and by painting a thin layer of celloidin over the block before each section it is not difficult to cut sections of 2 μ . Such thin sections are used only in studying the finer details of cell structure. A few segments were embedded in paraffine, which allows of even thinner sections and is a little more convenient for serial work. For general work I prefer the celloidin embedding. Unless otherwise stated, 6 μ is the standard thickness of section.

Section Cutting.—In addition to what has been said, I desire to emphasize the extreme importance of uniformity in thickness of sections. When one is dealing with such structures as those which make up the nerve cell, very different pictures are presented with varying thickness of section. It is therefore necessary in all comparative work, especially where the comparison is between normal cells and cells presumably pathological, to know the thickness of section and to compare only cells from sections of like thickness.

Staining.—For the greater part of the work the staining method used

is that of Nissl with modifications suggested by Held (6 and 20). Sections are first stained in a solution consisting of:

Erythrosin,	1
Aqua dest.,	150.

In this solution the sections are warmed for about 2 minutes, after which they are thoroughly washed in water and transferred to the following:

Methylene blue,	3.75
Venetian soap,	1.75
Aqua dest.,	1000.

To this is added after perfect solution an equal volume of a 5% aq. sol. of acetone. In this stain the section is heated until the acetone is driven off. It is then decolorized in a 0.1% aq. sol. of alum, washed in water, passed through alcohols and cleared in oil of cajeput. I have been accustomed to passing the specimens through alcohol after the erythrosin staining and washing with water, and think that a sharper picture is obtained by so doing. The specimen is then re-transferred to water before staining with methylene blue. I also prefer warm water for washing the specimens after both stains as there is less tendency to shrinkage of the specimen than when it is transferred from the hot staining fluid to cold water.

Of these rabbits, numbered 1, 2, 3, 4, 5, and 6, the following cells have been studied:

1. Stichochromes of the ventral horns and of the medullary nuclei.
2. Cells of the spinal ganglia.
3. Purkinje cells.
4. Mitral cells of the olfactory lobe.
5. Cells of the cerebral cortex.
6. Cells of the basal ganglia.

I. SPINAL AND MEDULLARY STICHOCHROMES.

Plate XXXIV, Figs. 1, 2 and 3.

In the cord these cells are found mainly in the anterior horn. Some occur in the central and posterior portions of the grey matter, and well out in the white matter along the course of nerve fibres. They are most numerous in the cervical and lumbar enlargements, and are arranged in what appear on cross sections to be ill defined

groups of cells, but which on longitudinal sections prove to be longer or shorter columns of cells. These groupings or columns are less distinct than in the human cord, and are not constant for different cords, for example, a section through the centre of exit of the second pair of lumbar nerves in one cord would not necessarily show the same groupings as a section through the corresponding locality of another cord. In the dorsal cord the cells are much fewer, and show but little grouping. In the medulla, these cells are found mainly in the *formatio reticularis*, which represents the breaking up of the ventral horn, and in the nuclei of origin of the cranial nerves. They vary greatly in size and shape, averaging 40 to 50 μ , being rather smaller than similar cells of the human cord.

A. The Nucleus and Nuclear Contents.—The nucleus lies approximately in the centre of the cell. It varies in diameter from 15 to 30 μ , averaging about 20 μ ; in general the larger cells have the larger nuclei, but there is less variation in size of nuclei than in size of cells.

The *nucleolus* is spherical, and is at the centre of the nucleus, or part way between the centre and the periphery. It varies in size from 2 to 6 μ . It stains intensely with the basic anilines, and when so stained may appear perfectly homogeneous (Plate XXXIV, Fig. 1), or may show one or several round or oval areas of lighter color, resembling vacuoles. While reacting more intensely to basic dyes, the nucleolus is far from unstainable with acid dyes such as eosin and erythrosin, which stain it bright red. Rehm (25) counterstaining with a solution of carmine 1 part, liq. ammon. caustic 1 part, in water 100 parts for five minutes, then decolorizing five minutes in alcohol 100 parts, potassium nitrate 1 part, stained the centre of the nucleolus red, while leaving a peripheral rim of blue. Ewing (26), in sublimate-hardened specimens treated with Ehrlich's triacid mixture, obtained the same result, the conclusion being that micro-chemically the nucleolus is composed of two parts, a central acidophilic, and an outer basophilic zone. Rehm also claims this reaction for only one intranuclear body, which he considers the true nucleolus, the so-called secondary nucleoli found in normal nuclei, and the fragments fre-

quently present in pathological nuclei, reacting to only the single dye.

I have been unable to confirm these claims of a double micro-chemical staining reaction for the nucleolus. The exact appearance of the nucleolus is dependent, to a large extent, upon the method of staining and the details of its application. As has been stated, when stained with methylene blue alone, the nucleolus takes a homogeneous or vacuolated dark blue stain; the same is true when a light erythrosin stain is followed by strong staining with methylene blue. The erythrosin stain can, however, be made so strong, that when it is followed by a weak or moderately strong methylene blue stain, the red color remains. Upon the border line lie those nucleoli in which an overstaining with erythrosin, or an understaining with methylene blue allows the central portion to retain its red color, while covering over the surface with the blue stain. This gives the appearance of a blue rim with a red or purplish red centre. By continuing the alcohol decolorization of such a specimen the blue rim may be removed, leaving the entire nucleolus stained red. In some of my specimens fixed in formalin and in van Gehuechten's fluid, sections mordanted in 5% aqueous solution of copper bichromate, and then stained by the Weigert-Pall method with subsequent rather under-decolorization, a quite different appearance was presented by some of the nucleoli. In a homogeneous nucleolar ground substance, clear or faintly yellow, are scattered fairly evenly a number of small, irregular, dark brown, or black granules. By a $\frac{1}{18}$ immersion lens extremely fine faint lines are seen to extend off from these granules, giving the impression of a stainable intra-nucleolar reticulum traversing a clear unstainable ground substance. That such a structure could exist, and yet be entirely undemonstrable by such intense dyes as erythrosin and methylene blue is certainly within the range of possibility. The nucleolus showed no variation in appearance due to differences in fixation.

The intra-nuclear network or nucleo-reticulum.—Eliminating the nucleolus, two nuclear substances remain, the one stainable the other non-stainable. The former reacts rather faintly to methylene blue, strongly to erythrosin. It consists of a reticulum, and of coarse and

fine granules. These granules, especially the coarser ones, tend to collect around the nucleolus and to extend out upon the strands of the network, thus surrounding the nucleolus with a somewhat stellate mass. There is no question as to the acidophile character of this reticulum and of the granules, for, although they can be stained, the reticulum faintly, the granules quite strongly with methylene blue alone, when both stains are used they invariably choose the acid dye. The meshes of the reticulum are irregular and vary in diameter, being smaller near the centre and larger near the periphery of the nucleus, where the reticulum becomes continuous with the limiting membrane. This membrane appears from its staining reaction to be of the same composition as the intra-nuclear network. On further analysis this reticulum and membrane are seen to be covered, at least throughout the greater part of their extent, by fine acidophile granules. Whether there is underlying these granules a true fibrillar reticulum, or whether the whole apparent reticulum is not merely the result of a stringing together of granules, many of them beyond the power of the microscope to reveal, I have been unable to determine.

The remaining ground-substance of the nucleus shows no evidence of any structure, and with the erythrosin-methylene-blue stain gives no reaction. In specimens which have been treated with a less selective dye than erythrosin, e. g. with a strong alcoholic solution of ordinary eosin, the nucleus often takes a rather diffuse stain. This may be due to an actual staining of the ground substance or possibly to a retention of the dye within the fine meshes of the reticulum.

Under the different methods of fixation used, the nucleus and nuclear contents presented a very uniform appearance. Formalin 5% and 10% and van Gehuchten's fluid brought out most clearly the intra-nuclear network. It was especially clear in rabbit No. 2, in which the nervous system was fixed in situ with formalin 10%.

B. The Cell Body.—(a). *The stainable substance of Nissl* is present in the form of irregular masses or clumps of granules. These masses are of various sizes and shapes, are rather coarsely granular and arranged in a manner generally parallel to the outlines of the

cell body and of the nucleus (Plate XXXIV, Fig. 1). Around the periphery of the nucleus they are apt to form a fairly complete ring of blue granules, thus emphasizing the nuclear contour. Near the centre of the cell these so-called Nissl bodies are densely packed together, thinning out somewhat towards the periphery where there is often an area entirely or partly free from this substance. This tendency to central concentration and a peripheral clear zone, I have found more marked in formalin-hardened specimens, especially when a 10% solution was used, as in rabbit No. 2. As a protoplasmic process is approached the masses become more spindle shaped, until in the process itself the granules are strung out in long narrow rods parallel to the long axis of the process (Plate XXXIV, Figs. 1 and 2, a). The amount of this stainable substance is here much less, in proportion to the non-stainable, than in the cell body proper. That part of the cell in immediate proximity to the axone is free from blue staining granules.

In stained sections cut 6μ and over in thickness, the Nissl bodies are dense blue, often quite homogeneous in appearance at their centres, and shading off to a lighter blue at the edges which always look granular. The thinner the section, the less extensive is the dense central portion and the larger is the peripheral granular area, and in sections of 2μ and under the entire mass is granular. In such sections and even in much thicker sections it is evident that the granules which make up one of these masses are not evenly distributed, being thickly packed in some places and in others farther apart. This gives sometimes a vacuolated appearance to a mass. Reference has been made (p. 554) to Held's description of a distinct embedding mass or coagulum in which the granules lie, this coagulum staining a violet color in contra-distinction to the blue of the basophile granules on the one hand and to the red of the acidophile element on the other. In none of the rabbit's nerve cells which I have studied, either normal or pathological, have I been able to make out any distinct embedding mass in which the granules are placed. That such a mass may exist is not at all improbable. When we consider that in the contents of nerve cells we are dealing with molecules of the highest complexity,

and hence of the greatest instability as well as potentiality, it would be rather surprising if there were not in these Nissl bodies, whatever their relation to the living cell, various constituents differing from one another in both chemical and physical features. In specimens stained with erythrosin alone the Nissl bodies take a red stain; when placed in the methylene blue solution, their greater affinity for that dye, or its greater intensity causes them to change to a blue color. My observations, however, have been that there is in this process a certain covering-over of one color by the other, and not an entire replacement of the red by blue; for, in most cases, if, after the staining with methylene blue, the decolorization be sufficiently pushed (especially if the specimen has been washed in alcohol after the erythrosin staining and water washing), the blue may be entirely removed and the Nissl bodies will again be seen stained pink or red. Again it is noticeable that at the surface of a chromophilic body, where the individual granules are separated from one another and more distinct, there is no appearance of a substratum of violet color, and that the more compact the mass of granules, the more clearly is the violet color seen. Furthermore, the shade, toward blue or toward red, can be varied at will by regulating the intensity of the methylene blue stain and the subsequent decolorization. It would thus seem that, while an embedding mass for the chromophilic granules, of different composition and staining reaction than the granules themselves, may exist, the appearances observed in these rabbit's cells can be explained on purely physical grounds.

In specimens stained with erythrosin and methylene blue, two constituents of the cell body remain after elimination of the chromophilic bodies: one of these is stained by erythrosin and hence denominated acidophile, the other remaining unstained we will speak of as the truly achromatic element of the cell.

(b). *The acidophile constituent* shows most clearly in specimens stained with erythrosin and methylene blue after fixation in formalin or van Gehuchten's fluid. It takes the form of fine anastomosing threads, giving the appearance of a distinct reticulum. In the parts of the cell where the basophilic element is most abundant, the ap-

pearance of the reticulum is largely obscured. It is seen to best advantage near the periphery of the cell and in the larger protoplasmic processes (Plate XXXIV, Fig. 2). Comparing it with Ewing's description and drawing of the reticulum found in the large stichochromes of the human ventral horn, the threads of this reticulum (rabbits' anterior horn cells) appear finer, the nodal points rather more marked. The size of the mesh is fairly uniform, averaging from 1 to 3μ in diameter. The shape of the mesh is irregular and the strands of the reticulum are sometimes quite smooth and again wavy, looking very much like the outline of omental endothelium as seen in silver-nitrate preparations. As the reticulum approaches a protoplasmic process, its meshes elongate, and in the processes themselves we have a reticulum with long, narrow irregularly rectangular meshes (Plate XXXIV, Fig. 2, a). This reticulum extends out into very fine dendritic branches, in fact I have failed to find any dendrites, however small, in which there was no trace whatever of the acidophile reticulum, though it was sometimes reduced to a single fibril. As the reticulum approaches the axis cylinder process, on the other hand, the appearance of an anastomosing network is gradually lost, by the disappearance of the connecting fibrils, the reticulum passing over into, and being continuous with, fine parallel fibrillæ which lie close together and compose the axis cylinder (Plate XXXIV, Fig. 2, c).

As to the ultimate structure of this reticulum, I am inclined to accept Held's idea already referred to, that it is made up of rows of staining granules upon the limits of microscopic perceptibility. With a Reichert $\frac{1}{18}$ immersion lens the appearance of the reticulum was not always altogether uniform. Some parts of the reticulum could be resolved into extremely fine but distinct granules; others looked like entirely homogeneous, anastomosing fibrils. The fact, however, that portions of the reticulum appear granular in structure, these granules being at the limit of microscopic vision with the highest powers, favors the hypothesis of a granular structure of the entire reticulum, many of the granules being so fine as to be unrecognizable as such.

The relation of the basophile granules to the acidophile reticulum

is seen to best advantage in extremely thin sections after fixation in formalin or van Gehuchten's fluid and staining with erythrosin and methylene blue (Plate XXXIV, Figs. 2 and 3). It shows most clearly in those parts of the cell where the basophile element is present in small amounts, e. g., at the periphery of the cell and especially in some of the longitudinal sections of the protoplasmic processes. Where the Nissl bodies are larger and more numerous, they extend over so many meshes of the reticulum and overlap each other in such a way as to obscure largely the acidophile network. The basophile granules seem to be laid down upon and around the strands of the acidophile reticulum, forming, as it were, a sort of incrustation (Plate XXXIV, Fig. 3). Each of the larger blue masses covers over several meshes of the reticulum and only at their edges can the fine rows of granules extending off into the delicate lines of the reticulum be seen. In extremely thin sections fine pink lines and irregular red spots (Plate XXXIV, Fig. 2, b) may sometimes be seen within the Nissl bodies, especially near their edges, which would seem to be strands and nodal points of the acidophile reticulum. In transverse sections of protoplasmic processes the long chromatic rods are seen in cross section and appear round, oval or somewhat irregular. These often show a minute pink dot (Plate XXXIV, Fig. 2, b), usually situated near the centre of the granule. This dot is sometimes so minute as to be barely visible; sometimes larger and more irregular. There may be several pink points instead of one. I believe that these pink dots represent strands of the acidophile reticulum in cross section and that the larger and more irregular bits of pink represent sections through nodal points of the reticulum.

(c). There remains that constituent of the cell which with the erythrosin-methylene-blue staining is entirely *achromatic*. The description already given of the achromatic part of the nucleus (p. 561) applies equally to the achromatic part of the cell body. It is unstained and apparently structureless. Certain other dyes, however, either stain this ground substance or are retained within the meshes of the reticulum.

I have repeated with some modifications the experiments of Held (6) in examining fresh nerve cells, with the following results:

The specimens consisted of bits of the ventral horns from the lumbar cords of rabbits and guinea-pigs. The animals were chloroformed, the bits of cord removed as soon as anesthesia began, and quickly teased apart in the following media, all at a temperature of 100° F.:

1. Solution of sodium chloride 0.6%. Nucleus and nucleolus distinctly visible; no evidence of any chromophilic bodies.
2. 0.6% sol. sodium chloride, to which was added sodium hydrate to 0.224%, or about the alkalinity of the blood. No chromophilic bodies seen.
3. 0.6% sol. sodium chloride, to which was added sufficient hydrochloric acid to have neutralized, volume for volume, the above alkaline solution. In this preparation indistinct irregular clumps of granules were visible which stained on the addition of methylene blue.
4. 0.6% sol. sodium chloride, to which was added methylene blue to 0.01%. No chromophilic bodies could be seen.
5. 0.6% sol. sodium chloride, plus sodium hydrate to 0.224% plus methylene blue to 0.01%. Result negative.
6. 0.6% sol. sodium chloride, plus hydrochloric acid sufficient to have neutralized an equal quantity of the above alkaline solution, plus methylene blue to 0.01%. This preparation showed distinct and well formed chromophilic bodies.
7. 0.6% sol. sodium chloride plus 0.1% methylene blue. Showed distinct chromophilic bodies.
8. 0.6% sol. sodium chloride, plus sodium hydrate to 0.221%, plus 0.1% methylene blue. Showed no chromophilic bodies.
9. 0.6% sol. sodium chloride, plus 1% methylene blue. Distinct chromophilic bodies.

The results may be summed up as follows:—

- (1). Nerve cells examined immediately after removal in warm normal saline solution neutral in reaction, in normal saline solution of approximately the alkalinity of the blood, and in the same plus a small amount of methylene blue (0.01%), showed nothing corresponding to Nissl bodies.
- (2). In warm normal saline of slightly acid reaction, an indistinct clumping of granules was observed, which stained on the addition of methylene blue and resembled chromophilic bodies.
- (3). In normal saline of slightly acid reaction plus methylene blue (0.1% or 0.01%), and in normal saline of neutral reaction plus methylene blue (0.1% or 1%), distinct chromophilic bodies were found.

Such observations tend to confirm the belief expressed by Held that the chromophilic bodies in the Nissl preparations represent merely the precipitation—the result either of post-mortem changes or of the action of fixatives—of a substance or substances previously held in suspension or in solution in the protoplasm of the living cell.

An attempt was also made to stain the cytoreticulum of the fresh cell with erythrosin, but with negative result. Such failure, however, does not disprove the existence of such a reticulum in the living cell, inasmuch as its structure is so delicate and requires such careful technique that it is difficult of demonstration even in hardened preparations. From observations upon both fresh and hardened tissues I am of the opinion that, whether existent in the living cell or not, whether a true reticulum or some such structure as that described by Bütschli, the strands of the acidophile mesh are formed before the chromophilic bodies, and that the latter are the result of a precipitation of fine granules which are caught in and upon the meshes of the reticulum.

2. NERVE CELLS OF THE SPINAL GANGLIA. *Plate XXXIV, Fig. 4.*

Ewing, in his "Studies on Ganglion Cells," sums up our knowledge of the human spinal ganglion cell, resulting from the investigations of von Lenhossék, Held, Flemming, Nissl, Cox, and Heimann, as follows: The cells vary from 60μ to 80μ in diameter, sometimes reaching 120μ . Each cell is enclosed in a connective-tissue capsule lined by a single layer of endothelium. In the normal living condition each cell completely fills the enclosed space. The cells are unipolar, the axone taking origin as usual from an achromatic area. Flemming describes three varieties according to the size and arrangement of the chromatic masses. Cox mentions two varieties—one in which small irregular chromatic masses are present without definite concentric arrangement, the other in which large irregular chromatic bodies are arranged concentrically. Ewing in human beings, and Heimann in the rabbit distinguish but a single type of spinal-ganglion cell, and with this my own observations coincide.

The cells of the spinal ganglia in rabbits (Plate XXXIV, Fig. 4)

vary in size from 20 to 80 μ . They are round or show sides flattened by pressure of contiguous cells. The nucleolus presents the same appearance as in the ventral horn cells. It is common for a nucleus to have two or more nucleoli. The intra-nuclear network is rather coarser than in the spinal stichochromes. The arrangement of the basophile substance varies so greatly as to have led some investigators to differentiate several types of ganglion cells. It always appears granular and the granules are as a rule quite coarse. They may be scattered fairly evenly throughout the cell with no arrangement into chromophile bodies (Plate XXXIV, Fig. 4, b), or there may be a peripheral zone or both peripheral and peri-nuclear zones, partly or entirely free from chromatic granules. In other cells the granules are collected into distinct masses, very irregular in shape, but having, in general, concavities towards the nucleus and convexities towards the periphery of the cell (Plate XXXIV, Fig. 4, a and a'). Taking the extremes of these cells, it is easy to construct a description of two distinct types of ganglion cells. Between these extremes, however, all gradations exist, and these, in view of the similarity in shape, the uniform unipolarity and the constant connective-tissue capsule, suggest minor variations, probably physiological, in a single type of cell, rather than several distinct types.

The cyto-reticulum in these cells is fine both as to its strands and size of mesh. It is usually clearly seen near the periphery of the cell but much obscured in the deeper parts. The relation of the basophile granules to the acidophile reticulum is very well demonstrated in some of these cells, and is the same as that described in the spinal stichochromes.

3. NERVE CELLS OF THE CEREBELLUM. *Plate XXXIV, Fig. 5.*

In the cerebellum, the only cells of special interest when subjected to the method of Nissl, are those of Purkinje. Formerly classed as arkyostichochromes, these cells Nissl now includes in the arkyochrome group. They lie as a distinct row of cells at the junction of the molecular layer with the granular layer, their cell bodies embedded in the latter. The point which makes them of most interest when

subjected to this method of staining, is their possible function as the motor cells of the cerebellum, presiding over the government of equilibrium. This and the fact that they seem to be rather quickly and easily affected by pathological conditions, adds interest to a close study of their normal appearances.

The nucleolus presents the same appearance as in the cells already described. It is not uncommon to find two, three, or even more nucleoli. The nucleus is small, in proportion to the size of the cell, round, and situated at the base of the cell. The intra-nuclear network may be either coarse or fine meshed.

The chromatic substance (Plate XXXIV, Fig. 5) is arranged somewhat like that in the spinal ganglion cells, i. e., masses of granules lying as longer or shorter curved rods with their long axes parallel to the contour of the nucleus and of the cell periphery, and their concavities directed centrally. The cells differ from those of the spinal ganglia in the wider separation of the Nissl bodies, and in the much closer packing of the extremely fine granules which make up a chromatic mass. These granules in many cases are so fine as to give the chromophilic bodies a homogeneous appearance. The chromatic substance is most abundant near the centre of the cell, becoming rapidly less towards the main dendrite. In most of these cell bodies, the cytoreticulum is indistinct, owing, often, to the diffuse staining of the ground substance. In the neck of the cell, and in the main dendrite, where the mesh becomes elongated, there is an extremely clear demonstration of the cytoreticulum and of its relation to the chromophilic bodies. There may be a well marked nuclear cap of chromophilic substance (Plate XXXIV, Fig. 5, b), and the chromatic pyramid at the bifurcation of the main protoplasmic trunk is quite large. Chromatic rods are found in the smaller dendrites as far as they can be traced in the section.

4. NERVE CELLS OF THE OLFACTORY LOBE. *Plate XXXV, Fig. 6.*

The accompanying diagrams, Figs. A and B, of the exterior of the rabbit's brain show the situations from which sections of the olfactory lobe and of the cerebral cortex were taken. The dimensions

are approximately normal for a medium-sized rabbit. The localization given is that of Mann. Speaking generally, this localization of function is probably fairly accurate, though most other experimenters have failed to make out quite as definite limits to the functional areas.

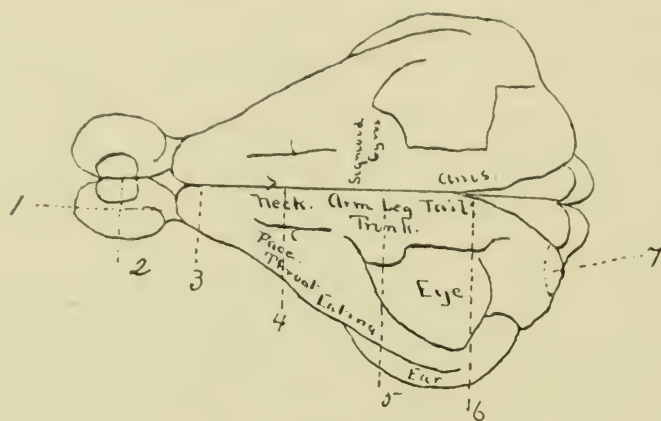


FIG. A.

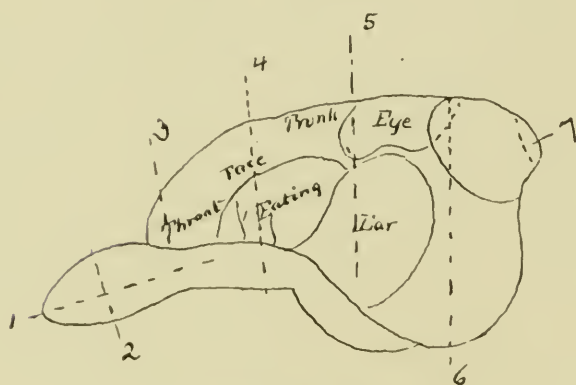


FIG. B.

FIGS. A and B.—Represent Gross Anatomy of Surface of Rabbit's Brain. Localization according to Mann.

FIG. A.—Rabbit's Brain. Dorsal view.

FIG. B.—Rabbit's Brain. Lateral view. Dotted lines show situations from which sections were taken.

The dotted lines of Fig. B represent points at which sections were taken. Owing to the very considerable difference in size of brain in large and small rabbits, these points were fixed by bisecting the distance between two fixed points, rather than by a definite measurement in millimetres from any one point.

Fig. C is a low power drawing, slightly diagrammatic, from an antero-posterior section through the middle of the olfactory lobe,

corresponding to dotted line 1, Fig. B, extending from the surface to the ventricle. The section was stained with methylene blue alone. It shows the layers of the olfactory lobe as given by Ramon y Cajal. Van Gehuchten examining these cells by Golgi's method interprets the appearances as follows:

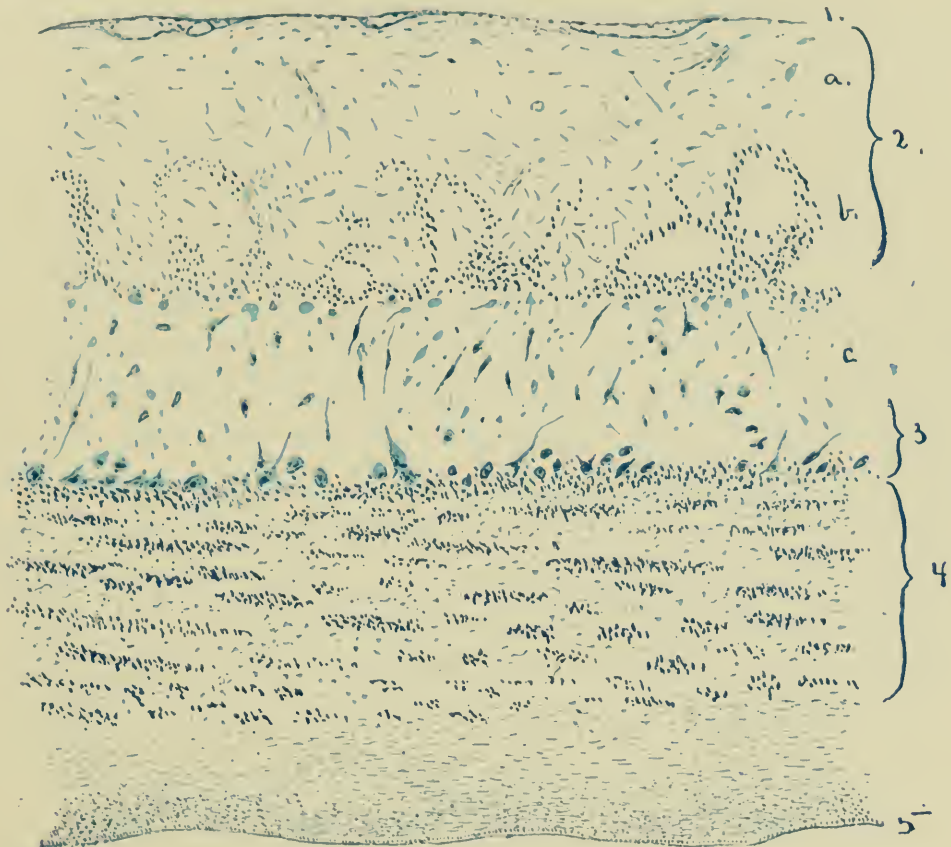


FIG. C.

Low Power Drawing, slightly diagrammatic, of Antero-posterior Section through Middle of Olfactory Lobe, corresponding to dotted line 1 of Fig. B, from surface to ventricle.

1. Pia Mater.
2. { a. Layer of Olfactory Fibres.
b. Layer of Olfactory Glomeruli.
- c. Inferior Molecular Layer.
3. Layer of Bipolar Cells (Mitral Cells).
4. Layer of Nerve Fibres and Granules: the Superior Molecular Layer.
5. Epithelial Layer.

1. Lying just underneath the pia mater is a layer formed by bundles of the peripheral olfactory nerve fibres. These are the axis-cylinder processes of the bipolar cells of the olfactory mucous mem-

brane. These axis-cylinder processes terminate freely, either directly or after bifurcation, in the glomerules.

2. Beneath this is a layer of grey matter rich in nerve cells. These are mostly of the bipolar type, and are called the layer of bipolar cells. At the junction of this layer with the next internal one, a considerable number of these cells are arranged in a row, much after the fashion of the Purkinje cells of the cerebellum. The axis cylinder processes of these bipolar or mitral cells pass downward into the white matter. Their protoplasmic processes terminate either freely or by arborization in the olfactory glomeruli.

3. An internal layer, formed by bundles of nerve fibres of the olfactory tract, intermingled with groups of small nerve cells.

From Fig. C' and the description just given, it will be observed that the great mass of the cells of the olfactory lobe are of the "granule" type, corresponding in appearance to the cells of the granular layer of the cerebellum. These are caryochromes and consequently possess little interest when stained by the method of Nissl. The layer of mitral cells, however, is composed of somatochromes which react characteristically to the Nissl technique.

The section from which the cells in Plate XXXV, Fig. 6, are taken, is a median transverse section of the olfactory lobe corresponding to dotted line 2, Fig. B. Both cells are situated in the line of bipolar cells marking the outer border of the internal granular layer. Cell a, Fig. 6, shows two processes, the one to the right passing downward into the granular layer, the one to the left passing toward the periphery. Both of the processes of cell b pass in the latter direction.

1. The nucleolus is usually eccentrically situated. It does not differ from the nucleoli already described. There may be more than one.

2. The nucleus is large in proportion to the size of the cell, is spherical or slightly ovoid in shape—in the latter case its long axis corresponding to the long axis of the cell—and is usually situated in the centre, more rarely near the inner surface of the cell. The intranuclear network is rather coarse, is well defined, stains strongly, and there is a rather large amount of granular acidophile substance just around the nucleolus.

3. The chromophilic bodies are of all sizes and shapes. They are coarsely granular, the granules being placed very closely, thus giving an almost solid appearance to the bodies unless the section be extremely thin or the bodies viewed at their peripheries. There are always present several large masses of chromatic substance, larger than those found in any other nerve cell, excepting those of the cornu Ammonis and some of the spinal and medullary stichochromes; they tend to be triangular, and to group themselves near the centre of the cell, around the nucleus. The "nuclear cap" is almost always well marked and a row of finer granules accentuates the outline of the nucleus. Besides these larger masses which vary in number, numerous smaller bodies are scattered throughout the cell. In the form of larger or smaller rod-like masses, the chromophilic substance extends out into the protoplasmic processes, usually as far as the section allows the processes to be traced. The chromophilic bodies of these cells stain intensely with methylene blue, and the rest of the cell body, being decolorized with considerable difficulty, often retains a faint blue tint.

4. The cytoreticulum is obscured in the central part of the cell by the large closely packed chromophilic bodies. Around the edges of the cell, however, and in the dendrites, it is clearly seen.

5. NERVE CELLS OF THE CEREBRAL CORTEX.

Plate XXXV, Figs. 7, 8 and 9.

The layers of the rabbit's cortex cerebri, as given by van Gehuchten, correspond to those of the human cortex.

1. The superficial layer, which has been called the barren layer or, more properly, the layer of few nerve cells, is made up almost entirely of non-medullated nerve fibres, which pass in a direction tangential to the surface. Many of these fibres are axis-cylinder processes of cells of the deeper layers; others are association fibres, being the cortical terminations of cells from other convolutions.

Scattered among these fibres are nerve cells, of which van Gehuchten recognizes three types: (a) A polygonal cell having four or five large protoplasmic processes, which pass in all directions, and an

axone, which, originating from the lateral aspect of the cell, runs parallel to the surface and terminates in this same layer. (b) A fusiform cell which lies horizontal to the surface, its long axis running antero-posteriorly. It has but these two dendrites which are so long that they are rarely wholly included in even a thick section. This cell usually has more than one axis-cylinder, and these spring from the dendrites at some distance from the cell body. The processes of this cell also terminate in this same layer. (c) A triangular cell with two dendrites passing laterally and a third passing downwards; there are several axones which spring from the dendrites.

2. Beneath the barren layer is one of small pyramidal cells. The axones of these cells pass downward to become medullated nerve fibres either of association or of radiation.

3. Without any distinct line of demarcation from the preceding, is a layer containing a considerable number of much larger cells of the same shape; this is the layer of large pyramidal cells. Their axones take the same course as those of the small pyramidal cells.

4. The layer of polymorphous cells. The axones of these cells pass, some downward, like those of the pyramidal cells, others upward to terminate in the cortex.

The regions of the cortex from which sections have been taken for study are indicated by the dotted lines in Fig. B (p. 570).

1. At line 3, about 2 millimetres back from tip of frontal lobe. Plate XXXV, Fig. 7.

The cells are mostly small and without any great variation in size between the layer of small pyramidal cells and that of large pyramidal cells.

The nucleolus is commonly eccentric and presents the same appearance as already described. Several nucleoli are often found. The nucleus is round or ovoid, rarely slightly irregular in shape. It is situated either at the centre of the cell, or in pyramidal cells near the base. The intra-nuclear network is well defined and rather coarse and stains dark red. There is apt to be a considerable amount of granular acidophile matter around the nucleolus.

The basophile constituent of the cell body varies greatly as to

amount and distribution in different cells. In most of the small pyramidal cells the collections of basophile granules are small, irregular in shape, and scattered very unevenly throughout the cell body. Plate XXXV, Fig. 7, b, shows a vertical section through such a cell, while Fig. 7, a, represents a section at right angles to the long axis. Most commonly one or two small chromatic masses are found, the remainder of the chromatic substance being in the form of fine separate granules which, lying on the strands of the acidophile reticulum, give the appearance of a blue network (Plate XXXV, Fig. 7, b and c). In most of the large pyramidal cells this same condition obtains. In some of the larger cells, however, are found Nissl bodies of more definite size and shape (Plate XXXV, Fig. 7, b). A large part of the cell body is often clear of chromatic substance. Rarely such a cell as Fig. 7, d, is found, in which a rather small nucleus is situated near the base of the cell and chromophilic bodies of fairly large and uniform size are distributed rather evenly throughout the cell.

The acidophile reticulum is extremely delicate, the mesh being small and the strands fine; otherwise it is identical with that found in other cells. The proportionately small amount of basophilic substance often allows the reticulum to stand out very sharply (Plate XXXV, Fig. 7, c).

The finer chromophilic bodies stain with methylene blue very lightly and are decolorized quickly. Even the larger chromatic masses of these cells show a disinclination to retain the dye, and, if the sections remain long in the aniline-oil alcohol, or even in strong alcohol, they are more or less completely decolorized.

2. At line 4 (Fig. B), equidistant from tip of frontal lobe and the line of mid-section.

The general arrangement of the cells here is the same as that just described. The cells of the small pyramidal layer are of about the same size as in the same layer of the preceding section. In the layer of large pyramidal cells, however, are many cells much larger than any found in the corresponding layer of the frontal lobe.

As regards the nucleolus, nucleus, and intranuclear network, the foregoing description applies. The nucleus varies greatly in size, bearing a fairly definite relation to the size of the cell.

The chromophilic bodies in these cells appear much the same as those shown in Plate XXXV, Fig. 7. The preponderance, however, of cells in which the chromophilic bodies are little more than granules, is no longer so marked. There are still, however, more of these cells than of any other type, especially in the superficial layer. Further down in the layer of large pyramidal cells the average size of the cells is much greater than in the corresponding layer of the frontal lobe, and there is a much larger number of cells fairly well filled with chromophilic bodies of considerable size and distinctness. Many of the larger cells, however, present but little chromatic substance in proportion to the size of the cell body. These large cells tend to arrange themselves in ill defined groups of from three or four to a dozen or more.

3. At line 5 (Fig. B). Transverse median section taken at an equal distance from the tip of the frontal lobe and the tip of the occipital lobe. Plate XXXV, Fig. 8.

The cells in this region of the cortex correspond somewhat to those already described. There are the same different types of cells as regards their chromophilic bodies, but a still further change in the numerical relation between them, and the cortical layers are better demarcated. The grouping of the large cells is more pronounced and the groups are larger than in the preceding sections. The cells are larger and their number proportionately much greater. In these large cells there may be a very small amount of basophile substance as in Plate XXXV, Fig. 8, a, and then the acidophile reticulum is distinct. In other large cells there is a considerable amount of chromatic substance and the granules are collected into quite large masses (Plate XXXV, Fig. 8, b and c). There are often very distinct nuclear caps.

4. At line 6 (Fig. B), equidistant from the tip of the occipital lobe and the mid-point.

There is again a preponderance, although not quite so marked as in the frontal lobe, of small cells, having very fine and indistinct chromophilic granules. In the layer of large pyramids there are fewer large cells, and these are scattered and show less tendency to grouping. Otherwise the description already given applies.

5. At line 7 (Fig. B). The tip of the lobe was removed and sections were then made through the tip of the prominence at right angles to a line tangential to the surface at that point. Plate XXXV, Fig. 9.

As we pass from the region last described, we find that there is here a decided diminution in the number of cells of the larger type. All of the cortex from the barren layer to the layer of large cells is occupied by extremely small cells. These cells have a small nucleus and a small cell body, sometimes a few fairly well defined chromophilic bodies, more often only a few fine granules.

The large pyramidal cells are much smaller than in the central regions of the brain, and are scattered irregularly and rather sparsely throughout the layer. Otherwise they resemble the large pyramidal cells of other parts of the cortex. Of the cells pictured in Plate XXXV, Fig. 9, those of type d greatly predominate in this region; c and b are found in moderate numbers, and type a is rare.

The results of this study of the rabbit's cortical nerve cells (Figs. 7, 8, and 9) indicate that certain types may be distinguished as follows:

1. The large pyramidal cell. This is found in all regions of the cortex, but the cells are much more numerous and of much larger size in the mid-region of the brain, that is about the region analogous to the fissure of Rolando in the human cortex—the general sensory-motor area. In this region these cells are in more or less definite groups. As we pass either anteriorly or posteriorly these large pyramidal cells decrease in both size and number, until in the frontal and in the occipital lobes they reach their minimum of size and frequency. These cells, in sections treated by Nissl's method, present three fairly distinct types, as regards the arrangement of their chromophilic bodies, these types, however, merging into one another without distinct dividing lines.

- (a). The large pyramidal cell in which the chromophilic bodies are small and few, the greater part of the cell appearing clear (Plate XXXV, Fig. 8, a). This is the predominating type of large pyramidal cells in all regions.

(b). The large pyramidal cell in which there is more chromatic substance in proportion to the size of the cell than in type (a) (Plate XXXV, Fig. 8, c, and Fig. 9, a). The chromophilic bodies are of considerable size, irregular in shape, and are largest in the region around the nucleus, where they form distinct nuclear caps. In these cells the chromophilic bodies, though quite numerous and of fair size, by no means fill up the cell, there being a fair amount of clear cytoplasm. They are distributed throughout the entire cortex but are more numerous in the mid-region.

(c). The large pyramidal cell in which the chromophilic bodies are quite large, and of fairly uniform triangular shape and size (Plate XXXV, Fig. 7, d, and Fig. 8, b). They are scattered rather densely and evenly throughout the cell body, giving the cell much the appearance of a motor ganglion cell of the spinal cord. These are the least frequent of all the cells of the cortex.

2. The small pyramidal cell. This is the predominating cell of the cortex. The arrangement of its chromatic substance presents two types.

(a). The small pyramidal cell in which there are only a few fine chromophilic granules scattered irregularly throughout the cell body, most of the cell remaining clear (Plate XXXV, Fig. 7, c, and Fig. 9, b, and d). These correspond to type (a) of the larger pyramidal cells. They are the most numerous cells of the cortex.

(b). The small pyramidal cell, containing several chromophilic bodies of fair size, corresponding in general arrangement to type (b) of the larger pyramidal cells (Plate XXXV, Fig. 7, b, and Fig. 9, c).

3. Irregular cells scattered throughout all parts of the cortex, but most numerous and largest in the deeper layers, and in the mid-region of the brain. They vary greatly as to size and arrangement of their chromatic elements, from a few scattered granules to large regular chromophilic bodies resembling those found in the spinal stichochromes.

6. NERVE CELLS OF THE BASAL GANGLIA.

Nucleus Caudatus.—The majority of the cells of this nucleus are small multipolar elements possessing no characteristics distinguishing

them from similar cells found in other parts of the nervous system. They vary in diameter from 10μ to 20μ . The nuclei are round or slightly oval in shape, with, as a rule, a single centrally placed nucleolus. In the larger of these cells the nucleus is surrounded by a considerable area of cell body, in the smaller the cell body is reduced to a mere rim surrounding the nucleus. The acidophile reticulum is extremely fine-meshed but presents the same appearance as in cells already described. The amount of the basophile substance present is extremely variable, as is also its distribution within the cell body. In most of the cells the amount is rather small and the granules lie upon the strands of the reticulum. In cells where more of the basophile element is present it is apt to accumulate along the outside of the nuclear membrane, at the periphery of the cell, and as irregular clumps of granules near the origins of the main dendritic processes.

Among these smaller cells are scattered a few much larger ones corresponding in type to the spinal stichochromes. They seem to differ in no way from the smaller ventral horn cells.

There is also a considerable number of cells in which the basophile substance is present as fine granules giving a blue dusty appearance to the cell body. These cells Nissl describes as "gryochromes."

Nucleus Lenticularis.—The cellular elements of this nucleus resemble those just described in the nucleus caudatus. In the sections which I have examined the small nerve cells average rather larger and the large stichochromes are larger and somewhat more numerous than in the caudate nucleus. — In both nuclei the closeness with which the small cells are packed together and the number of large cells which are scattered among them vary greatly in different parts.

Optic Thalamus.—This region of the basal ganglia presents an extremely complex arrangement of its nuclei. The differentiation of the thalamic nuclei of the rabbit has been carefully worked out by Nissl, who distinguishes:

1. An Anterior Nucleus. This is situated in the most anterior part of the thalamus, and is separable into a larger part—the anterior ventral nucleus, and a smaller part—the anterior dorsal nucleus. In

the lower and outer portion of the anterior ventral nucleus, the cells have a quite compact arrangement and allow the differentiation of a lateral ventral, from a median dorsal area.

2. A Medial Nucleus. This nucleus lies near the median line, in much the same position as that occupied more anteriorly by the anterior nucleus. From the main body may be separated off a small anterior segment or anterior medial nucleus, leaving a larger remaining nucleus lying near the median line and extending about one-half the length of the thalamus.

3. Nuclei of the Reticular Zone. These are divisible into an upper dorsal part lying just below the anterior ventral nucleus, and a lower ventral portion. To the outer side of the latter is a small nucleus, the lateral nucleus of the reticular zone.

4. A Nucleus of the Middle Line. This is a flat plate of cells lying near the middle line. The projection inward of the main portion of the medial nucleus divides this nucleus throughout the greater portion of its extent into a dorsal and a ventral part.

5. A Lateral Nucleus. This is divided into a larger anterior part, extending through about two-thirds the length of the thalamus, and a smaller posterior part.

6. A Ventral Nucleus. This contains three main groups of cells, designated according to their location as the dorsal, the medial, and the lateral divisions of the ventral nucleus.

7. A nucleus lying close to the *tænia thalami*.

My studies of sections through the thalamic regions have been entirely confirmatory of those of Nissl. The main nuclear masses are easily distinguished, especially if followed from section to section. Isolated sections are sometimes confusing owing to the fact that at some point or points nuclei run into one another without any distinct demarcation. This is true especially of the posterior lateral nucleus, which at various points is continuous with the neighboring nuclei; for the same reason, there is sometimes difficulty in differentiating the subdivisions of the main nuclei. Again, neither shape, size, nor position of the various nuclei is absolutely fixed, there being some variation, usually slight for different rabbits.

No new types of nerve cells present themselves in these nuclei. The majority of the cells are of the small multipolar variety, resembling those found in the caudate and lenticular nuclei. There is not, however, that diffuse scattering of large stichochromes found in the nuclei of the corpus striatum. These large cells of the type of the anterior horn cells, are found mainly in the postero-lateral nucleus, which is made up mostly of large cells, the largest found in the thalamus. The median ventral nucleus also contains comparatively large cells. In the nucleus of the middle line and in the lateral ventral nucleus the cells tend to the fusiform variety. As is common in this form of cell, the basophile element is present mainly at the ends of the nucleus directed towards the main dendrites.

II.

CHANGES IN THE NERVE CELLS IN RABIES PRODUCED BY SUBDURAL INOCULATION OF RABBITS WITH FIXED VIRUS.

PLATES XXXVI-XXXVIII.

HISTORICAL REVIEW.

Although it is not the purpose of this article to consider the pathology of rabies further than the presentation of personal observations, mostly relating to changes in the nerve cells of rabbits inoculated beneath the dura with fixed virus, it will not be out of place to present a brief review of our knowledge of the pathological anatomy of this disease.

Observations upon the post-mortem appearances in rabies date back to the essay of Richard Mead (32) in the beginning of the eighteenth century and to the writings of van Swieten (33) later in the same century. They found no lesions in any way characteristic of the disease. Nor in the many recorded autopsies since these writers have characteristic gross lesions been detected. Congestion, œdema and small hæmorrhages in various parts of the central nervous system and degenerations such as are common to many infections have been noted. The claims of Gamaleia (34) and Schaffer (35) that macroscopic areas

of softening and necrosis in the spinal cord are characteristic of rabies have not been substantiated.

Even before the important discoveries of Pasteur, and more positively since these, it has been clear that the essential seat of rabies is in the central nervous system, and consequently there have been many careful microscopic studies of this part in recent years. These have brought to light the existence of lesions, mostly of a focal or miliary character, connected partly with the blood-vessels and partly with the nerve cells, the former being the ones chiefly emphasized until quite recently. Whether or not these lesions be regarded as in part characteristic, it has been established that rabies is no longer to be ranked among diseases without a definite and demonstrable anatomical basis.

The most important of the vascular lesions, as was pointed out a quarter of a century ago by Benedikt (36), Kolessnikow (37), Coats (38) and Gowers (39), is an accumulation of leucocytes around the blood-vessels, particularly in the spinal cord, medulla oblongata, and basal ganglia. Other vascular lesions frequently found are congestion, irregular dilatations, thrombosis, hæmorrhages, especially within the perivascular lymph-spaces, the deposit of hyaline both within and around vessels, and swelling of the endothelial cells.

The accumulation of leucocytes is not limited to the immediate neighborhood of the blood-vessels. They may appear around the central canal and in the nervous tissue, and especial importance attaches to their accumulation around nerve cells, particularly the motor ganglion cells, as pointed out by Babes (40) and Schaffer (35). To these pericellular and perivascular focal accumulations of small round cells, more especially to the former, Babes has given the name of "rabie tubercles" or nodules, and he considers that these are so characteristic of rabies that, when due attention is given to their intensity and distribution, they can serve as a diagnostic criterion of the disease.

In addition to these exudative hæmorrhagic lesions referable to the blood-vessels, various changes, mainly of a degenerative nature, have been found in the nerve cells both of the cerebro-spinal axis and of the ganglia. Schaffer described atrophy, vacuolation, and granular, hyaline and fibrinous degenerations of nerve cells associated with degeneration of the processes and hyperplasia of the neuroglia. Confirmatory and additional observations have been made by Babes, Gianturco (41), Golgi (42), Germano and Capobianco (43), and others. Thus Golgi, working with his silver method, has described irritative and regressive changes in the nucleus, characterized by increase and subsequent fragmentation of the chromatin with karyolysis, bladder-like swelling, vacuolation

and granular degeneration of the cell-body, atrophy and loss of the processes, and eventually destruction of the entire cell. The most extensive alterations, terminating in complete disappearance of nerve cells, were noted by Germano and Capobianco. Only certain cells were affected, others in varying numbers and distribution remained normal.

It is to be emphasized that the lesions described were observed, at least in a marked degree, mainly in street rabies. Babes says that in rabbits inoculated with fixed virus there is usually no lesion of the nerve cells—a conclusion not in accord with my observations.

The lesions described have been usually interpreted as manifestations of a disseminated exudative and parenchymatous encephalomyelitis.

Such in brief outline was the general state of our knowledge of this subject when there appeared early in 1900 the notable papers of van Gehuchten and his pupil, Nelis (44), which have stimulated other contributions. This later literature has been the subject of an admirable critical review by Crocq (45). These recent studies have drawn attention to an almost forgotten observation of Pollaillon and Nepveu (46), who in 1872 described in a case of human rabies the accumulation of round and oval cells around degenerated nerve-cells in the Gasserian ganglion.

Van Gehuchten and Nelis contend that the vascular and cellular changes described by previous investigators, including the “rabie tubercle” of Babes, are inconstant and secondary, not characteristic of rabies and unimportant for diagnosis of the disease, whereas the rabie pericellular nodules in the cerebro-spinal and sympathetic ganglia are so pathognomonic as to constitute a valuable means of rapid diagnosis of rabies. These latter nodules consist of epithelioid, oval and round cells, derived partly from proliferation of the capsular endothelium, partly from other fixed cells, and partly from the blood-vessels, and accumulated around and eventually invading and displacing the ganglion cells. In their first publications these authors believed that they had found the long sought specific lesion of rabies, but van Gehuchten is now of the opinion that this lesion characterizes only the natural disease in man and animals, and is either absent or only imperfectly manifested in the experimental disease, at least by the ordinary modes of inoculation, whether by virus of the streets or fixed virus. The ganglionic lesion is more marked in dogs than in man, and least developed in rabbits.

The findings of van Gehuchten and Nelis have been in the main confirmed by Hébrant (47), Nocard (48), Cuillé and Vallée (49), and

Ravenel and McCarthy (50), so far as the constancy of the lesion in dogs dead of the natural disease is concerned. The search for the lesion in experimental rabies has given apparently conflicting results. Whereas Ravenel and McCarthy obtained positive results even in rabbits dead of subdural inoculation with the street virus, van Gehuchten (in his later publications) and Hébrant were unable to find the lesion either in rabbits or in dogs after subdural inoculation, these latter animals, as already stated, presenting the most advanced and characteristic changes after death from the spontaneous disease. There are not as yet sufficient observations to judge of the frequency of the lesion in human beings. It was well marked in a case reported by Gratia (51), but was absent or at most only slightly apparent in Sano's (52) patient.

It appears from later investigations that the ganglionic lesion described by van Gehuchten and Nelis is not, as they at first supposed, specific for rabies, but may occur in other diseases. Babes, who protests vehemently against the exclusive position taken by van Gehuchten and Nelis, says that he has met and described this alteration in several diseases, and essentially similar changes have been recently reported in various affections by Crocq (45), de Buck and de Moor (53), and Spiller (54).

It would appear, therefore, as stated by Crocq, that, while no lesion specific for rabies has hitherto been discovered, the totality of the lesions, both those emphasized by Babes and those described by van Gehuchten and Nelis, makes a picture sufficiently characteristic to warrant usually the diagnosis of rabies, when all of the circumstances of the case are considered. The absence of these lesions does not, however, exclude rabies.

MATERIAL AND METHODS.

The material used in the present study of the nerve-cell changes in rabies was obtained from nine rabbits which had been inoculated with fixed virus, an emulsion made from the medulla of a rabbit from a previous series being used. The skull was trephined and the injection was made under the dura. The strength of the virus was such that it proved fatal to control rabbits in from seven to nine days.

Rabbit No. 1. Killed by chloroform three days after inoculation. This rabbit showed no symptoms, was eating well and was apparently in a perfectly normal condition.

Rabbit No. 2. Killed by chloroform four days after inoculation, on

first sign of symptoms. These consisted merely in slight disinclination to eat and in lessened activity.

Rabbit No. 3. Killed by chloroform five days after inoculation. Partial paralysis for about 24 hours.

Rabbit No. 4. Killed by chloroform six days after inoculation. Partial paralysis for about 36 hours.

Rabbit No. 5. Died six days after inoculation. Was taken sick much earlier [second day] than the other rabbits and differed from them in showing much greater activity.

Rabbit No. 6. Died on seventh day after inoculation. Paralysis was almost complete. Had showed symptoms for about 60 hours.

Rabbit No. 7. Died on eighth day, four days after initial symptoms. Paralysis complete.

Rabbits Nos. 8 and 9. Died on ninth day. Paralysis complete.

The rabbits belong to two series, rabbits Nos. 2, 4, and 9 belonging to one, while rabbits 1, 3, 5, 6, 7, 8 belong to a second series. Most of the material was fixed in van Gehuchten's fluid (see p. 557) followed by strong alcohol. In some cases alternate segments were fixed in formalin 10%, followed by graded alcohols.

The sections were of a standard thickness of 6μ . For special purposes a few thinner sections were made as noted. The staining was mainly by the erythrosin-methylene-blue method described on page 558. A few sections were stained with methylene blue alone.

PERSONAL OBSERVATIONS.

RABBIT NO. 1.—*Spinal Cord and Medulla.* There is a marked congestion of both grey and white matter throughout the entire cord and medulla. There are no changes in the vascular walls, no exudation, hæmorrhages, or increase of neuroglia.

Cells of the anterior horn.—The majority of the spinal stichochromes appear perfectly normal. Of cells which show departure from the normal, we observe:

1. Cells, with normal nucleus and cytoreticulum, in which the chromophilic bodies, although normal in size and shape, present ragged, frayed-out edges, an appearance quite different from that of the chromophilic bodies found in the normal cell. There is no breaking up of the bodies, no lessening of intensity of staining, no diffuse staining of the cell protoplasm (Plate XXXVI, Fig. 10, a).

2. Cells with normal cytoreticulum, in which the chromophilic bodies, while normal in size and shape, in addition to the ragged appearance mentioned, take a much paler stain (Plate XXXVI, Fig. 10, b). In these cells the nucleus and nuclear contents remain normal, or the nucleus is somewhat swollen. Whether normal in size or swollen, its reticulum is apt to show an increase in the thickness of its strands, which thus appear more sharply defined. The same is true of the nuclear membrane, which thus gives to the nucleus a more distinct contour.

3. Cells in which, with or without the above nuclear changes, there is a marked reduction in the chromatic element.

(a). The Nissl bodies may retain their normal size and shape, but, in addition to the roughness of outline, appear full of ragged holes or vacuoles. On closer examination these are seen to correspond to the meshes of the cytoreticulum (Plate XXXVI, Fig. 10, c).

(b). Through further reduction in the chromatic substance the shape of the Nissl bodies is lost, and the basophilic element appears as fine granules incrusting more or less thickly the strands of the acidophile reticulum. At nodal points of the reticulum there are apt to be larger accumulations of granules. Not all strands of the reticulum are covered with the granules, nor do those covered have an equally thick incrustation at all points. The result is often to give the cell the appearance of having two distinct reticula, a fine meshed, acidophile reticulum, and a much coarser meshed, irregular, basophile reticulum. The chromatic rods or spindles in the dendrites, and in the cones which mark the dendritic bifurcations, do not show the same tendency to disintegration as the chromatic masses of the cell body proper. Even in those cells in which chromatolysis was most marked, these spindles and cones retained their smooth outline, finely granular appearance, and normal staining qualities. Such cells are found throughout the entire cord and medulla. The changes are, however, more pronounced, and involve a larger number of cells, in the cervical and upper dorsal, than in the lower dorsal and lumbar regions.

The stichochromes of the *medullary nuclei* show a larger propor-

tion of affected cells, and a larger proportion of these show greater loss in chromatic substance, than the cells of the cord. In the lower part of the medulla, where the grey matter of the anterior horns becomes broken up by decussating fibres, extremely large cells, of the type of the anterior-horn cells, are found scattered singly or in small groups. A large proportion of these cells are affected, and many show an almost complete disappearance of the chromophilic bodies. There is no selection of cranial nuclei, all being quite evenly affected; nor is there in the cord any tendency to degeneration of cells by groups; cells in the various conditions described lying in close proximity to one another and to normal cells. Speaking generally, those cells lying in the ventro-lateral portion of the horn, near the margin of the grey matter, show least changes, while those lying deeper and more internal, are most affected. Plate XXXVIII, Fig. 22, shows one of the more changed cells from the deeper part of the grey matter, near the central canal, in the upper cervical margin. Of changed cells, those described under 1 are much the most numerous, while those under 2 and 3, a and b, are in the order of decreasing frequency.

The cells of the *spinal ganglia* show no variation from the normal.

The *cells of Purkinje* show nothing beyond the usual wide variation in quantity and arrangement of the chromatic substance.

In the *cortex* and in the mitral cells of the *olfactory lobe*, no changes could be discovered.

Basal ganglia.—In the nuclei of the basal ganglia, the changes resemble closely those described in the spinal cord, corresponding as regards number of cells affected, and extent, to the upper or middle cervical region. There seems to be a decrease in intensity of the lesion as we pass in either direction from the medulla oblongata. In the larger ganglion cells scattered throughout the nucleus caudatus and the nucleus lenticularis, and in those making up the bulk of the postero-lateral nucleus of the thalamus, the changes are identical with those described in the spinal stichochromes.

RABBIT No. 2.—*Spinal Cord and Medulla.* The congestion is more marked than in the preceding animal, and extends throughout

cord and medulla. The walls of some of the vessels are infiltrated with leucocytes, sometimes many and sometimes few. These vessels may show also an increase in the connective tissue elements of the wall, most marked in the small vessels of the grey matter, the walls of which are normally very thin. Less frequently the latter condition is found without the leucocytic infiltration.

Hæmorrhages are common both in grey and in white matter. They are usually small, but sometimes are extensive, especially those in the grey matter of the anterior horn. They are always without any distinct limitation.

There appears to be no direct relation between the vascular changes and the cellular lesion. Normal cells are as common in the areas of most intense congestion and in the vicinity of vessels in the walls of which the most changes are found as elsewhere. I have seen apparently normal cells lying in the midst of a large hæmorrhage.

Cells of the anterior horn.—The cellular lesion is much more pronounced than in Rabbit No. 1. There are fewer normal cells, although these are still in the majority. The number of cells showing the lesser degrees of change, as described for the preceding cord, is increased. There is the greatest relative increase, however, of those cells which show the more marked chromatic changes.

The nucleus and nuclear contents are normal, or show only those slight changes already described. By far the majority of altered cells present only the lesser degrees of change already described, but there is often a much more diffuse granular blue staining. This obscures the cytoreticulum and gives the cell a quite homogeneously granular appearance. The cytoreticulum in those cells in which it is not lost in the diffuse blue granulation, appears normal.

A few cells show evidence of a more advanced condition of degeneration. Such cells, found in the cord, but more commonly in the medullary nuclei, or in the remnants of the anterior horn, show the following changes: For convenience of reference we will designate them as belonging to type 4, the changes already noted in Rabbit No. 1 having been referred to types 1, 2, and 3, a and b. The nucleolus, sometimes normal, is more often roughened at its edges, some-

times small pieces being broken off from the main mass. The intranuclear network is broken up, often appearing only as a stellate mass of granules around the nucleolus and as little thread-like projections from the inside of the nuclear membrane. The nuclear contour may be very distinct, and with its normal spherical shape; less commonly it is irregular, or somewhat crenated. There may be a break in the continuity of the nuclear membrane, in which case the nucleolus, though usually remaining within the nucleus, may be outside what appears to be the nuclear limits.

The Nissl bodies are broken up into small fragments, which usually lie upon nodal points of the reticulum. Between the fragments there is a fine, blue, granular deposit. The larger fragments may be absent and only the fine granules remain. The chromatic rods in the dendrites retain their normal appearance. The cytotreticulum, even in these cells, appears normal. The entire cell is often swollen, sometimes sufficiently to exert pressure upon the surrounding structures. More rarely it is shrunken, leaving an exaggerated pericellular lymph space.

The distribution of the lesion corresponds to that given for Rabbit No. 1, except that there is less difference between the upper and the lower regions of the cord. As in No. 1, the medullary nuclei and the cells of the anterior horns are especially affected.

The cells of the *spinal ganglia* are normal.

In the *cerebrum* and *cerebellum* the congestion is less marked than in the cord, and the changes in the vessel walls are absent.

There is a decided reduction in the average amount of chromatic substance in the cells of Purkinje, but many appear normal. Nuclear changes are seen in but few cells, and these are of the milder grade, as described in Rabbit No. 1. Some cells of Purkinje show a considerable amount of chromatic substance, but in a much finer state of subdivision than normal; others show complete, or almost complete, loss of the basophile element, with a diffuse blue ground-substance, a finely granular blue ground-substance, or the same with a few large granules. Both their behavior and their relative numbers would indicate that the former change is a result of degeneration of those

Purkinje cells which are normally rich in chromatin; while the latter results from changes in cells which are normally deficient in this constituent.

No changes were found in the cells of the cerebral cortex, or in the mitral cells of the olfactory lobe.

Basal ganglia.—The large stichochromes of the nucleus caudatus and the nucleus lenticularis, and the similar cells found in the nuclei of the thalamus, present the same degrees of degenerative changes as described for the spinal stichochromes. Rather more cells are affected, and the lesion of the individual cell is somewhat more advanced than in Rabbit No. 1. In the smaller ganglion cells there is an evident decrease in the basophile element. These cells differ from one another so greatly, as regards their normal content of basophile substance, that it is difficult to say of any particular cell that there is a diminution. When, however, the chromatic substance of a number of cells is estimated, it is apparent that the total average amount is considerably decreased.

RABBITS NOS. 3 AND 4.—The lesions found in Rabbit No. 3 and in Rabbit No. 4 resemble each other so closely that they may be described together. No. 3 was killed on the 5th and No. 4 on the sixth day after inoculation. They belong to different series (p. 585).

Spinal Cord and Medulla.—There is an increase both in the congestion and in the leucocytic infiltration of the walls of the blood-vessels. These leucocytes may form an even ring around the vessel, or, more commonly, there is a large collection of them at one or two points in the vascular walls. There seems to be no advance in the increase of connective-tissue elements, that feature remaining the same as in Rabbit No. 2. Hæmorrhages also are about as frequent as before.

Lesions of the nerve cells show a marked advance. Normal cells are still found, but in decided minority, so that search through several sections at any level of the cord may be required in order to find an entirely normal cell. They are still present in those regions where they have already been described as most frequent, namely, in the outer and ventral corner of the horn, and with decreasing frequency

from below up. All of the cellular degenerative conditions, previously described, are present, and are increasingly frequent. The relative increase is, however, greatest in cells showing the more extensive changes. Thus cells of type 3 a and b (p. 586) and type 4 (p. 588) are now quite numerous. Of further changes the following were noted:

Nucleus and nuclear contents.—(a). The nucleolus is usually rough, stains more irregularly than heretofore, appears rather coarsely granular, and is often broken up into several pieces (Plate XXXVI, Fig. 11).

(b). The nuclear reticulum is in many cells abnormally distinct and darkly stained, with larger mesh openings, due to a coalescence of some of the strands of the reticulum. This may stand out against a perfectly clear background, or one in which there is more or less granular or homogeneous pink color. There may be no sign of a reticulum, the entire nucleus staining a homogeneous, an evenly granular, or an irregularly granular pink color. The nucleus may assume almost any shape, is commonly very much shrunken and crenated, when it appears as an irregular red mass surrounding the nucleolus (Plate XXXVI, Fig. 11).

(c). The nuclear membrane is often broken, sometimes showing only in spots, again having completely disappeared; in this case the nucleolus appears to lie in the general cell protoplasm, with or without some remnants of the acidophile nuclear elements attached to it. In any of these conditions the nucleus may be eccentric.

Cell body.—(a). The basophile element of the cell body shows all of the types of changes already described. The most frequent condition is that described in Rabbit No. 1, as type 3, a and b, in which the basophilic granules appear as a precipitate upon the strands of the cytoreticulum. This gives the appearance of a densely granular, blue network with coarse strands and small mesh openings (Plate XXXVI, Fig. 11). In places where the granules are absent, or thin, the acidophile reticulum shows plainly. In contrast to those cells described in Rabbit No. 1, type 3, b, in which the chromatic substance is present in fine granules, and the chromatic spindles and cones in the

dendrites are intact, these cells in which the chromatin is arranged as a network, rarely show any normal condition of the dendritic chromophilic bodies, these being usually absent (Plate XXXVI, Fig. 11).

(b). The cytoreticulum appears intact, excepting in cells where there is considerable diffuse staining. In such cases it seems probable that the reticulum is obscured rather than destroyed.

(c). The outline of the cell body is often broken and irregular. The cell is more commonly shrunken than swollen or normal in size. These cells have the usual number of protoplasmic processes, many of which, however, are shrunken, stain dark red, and end in a point at a short distance from the cell body. Plate XXXVI, Fig. 11, illustrates quite typically several of the points described.

The distribution of the lesion is more uniform than before throughout the cord and medulla, being almost as extensive in the lower as in the upper cord and in the medulla.

The cells of the spinal ganglia show no changes.

Purkinje's Cells.—These are much more altered than in the preceding rabbits.

Nucleus and nuclear contents. (a). The nucleolus is usually ragged, sometimes broken up into several pieces. Eccentricity is often extreme, in some cases the nucleolus lying on the nuclear membrane.

(b). The nucleoreticulum is more or less completely disintegrated, and represented by clumps of granules surrounding the nucleolus and attached to the nuclear membrane. These granules are much coarser than those resulting from the disintegration of the nucleoreticulum in the anterior horn cells. There is almost no tendency to diffuse staining of the nucleus, as seen in some anterior horn cells.

(c). The nuclear membrane is in most cells very sharply defined and darkly stained, and preserves its spherical shape: more rarely it is crenated or broken or both. These latter conditions are not nearly so frequent as in the spinal stichochromes of this same rabbit.

Cell body. The various conditions of the cell, as regards the chromatic elements, are quite similar to those described in the cord and medulla, bearing in mind the normal differences. There are

some normal cells. A considerable number of cells show complete chromatolysis, the cytoreticulum being very distinct and apparently normal. Between this and the norm all grades exist. Two features of the chromatolytic process observed in the anterior horn cells, were absent in the cells of Purkinje. First, there was no appearance of fraying out of the edges of the Nissl bodies, as is the case in the anterior horn cells; they seem rather to disintegrate, breaking up into smaller, but no less dense and smoothly outlined masses. Second, there is no such distinct formation of a basophile network as in some of the anterior horn cells.

Staining of the ground substance is extremely irregular; it may be clear, or blue and granular, or homogeneous. There may be no blue staining whatever, the cytoreticulum then appearing very distinct against a clear background, or all or part of the cell may present a granular or homogeneous pink appearance. The cell contour is rarely broken, but may be irregular or shrunken.

Cerebral cortex. The congestion is not marked as in the cord; some of the vessel walls show exudation of leucocytes, but this also is less marked than in the cord.

The distribution of the cortical lesion is uniform, all types of nerve cells in all situations showing modifications. In the smaller pyramidal cells, and especially in those least rich in chromatic substance, earlier changes are difficult to study. In larger cells, both pyramidal and polymorphous, there is a disappearance of the larger masses of chromatic substance which lie upon the nodal points of the acidophile reticulum, with a resulting added clearness of the network-appearance of the basophile granules which lie upon the strands of the reticulum. Plate XXXVIII, Fig. 23, illustrates some of the conditions found in these cells. The changes in the smaller pyramidal cells are the same as in the larger; disappearance of chromatin is apt to be more complete. The large polymorphous cells of the deeper layers of the motor areas of the cortex show about the same degree of changes as those described in Rabbit No. 1 for the anterior horn cells. Few show more than a raggedness in the outline of the chromophilic bodies.

Mitral cells of the olfactory lobe. Most of the cells show changes. It will be remembered that extremely large, finely granular, dense

chromophilic masses arranged parallel to the nuclear outline and to the surface of the cell, are characteristic of these cells (p. 573), the nuclear cap being usually very large. These masses are usually packed closely, leaving few unstained portions of the cell, and largely obscuring the cytoreticulum. In most of the cells in Rabbits Nos. 3 and 4, these large chromophilic bodies are entirely or partly broken up into various sized masses and granules, distributed unevenly throughout the cell body. Some cells show no large Nissl bodies; others show but one or two, while the rest of the cell body is occupied by small masses and fine granules. The acidophile reticulum, which in the normal cell is barely visible between the dense chromatic masses, is now quite clear. There is little tendency to diffuse staining of the cell background (Plate XXXVI, Fig. 12).

The nucleolus is usually ragged or broken up. The nuclear reticulum is often somewhat disintegrated, though its strands are often quite distinct. The outline of the nucleus is smooth, rarely irregular, and the nucleus remains approximately in the centre of the cell.

In the case of all the cellular lesions previously described, speaking generally, there is some relation between the extent of nuclear changes and that of alterations in the body of the cell. Thus the more advanced nuclear changes are found usually in cells in which the greatest changes have taken place in the chromatic elements. This is, however, not always the case. Advanced nuclear disintegration is rare in cells showing mild grades of chromatolysis. On the other hand it is not at all uncommon to find a nearly normal nucleus in the midst of marked changes in the cell body.

Basal ganglia. Few of the larger cells of the nuclei of the corpus striatum and thalamus are normal. The extent of the lesion corresponds almost exactly to that in the upper part of the cord, both as regards the number of cells affected and the character of the lesion in the individual cells. Many of the cells present the condition illustrated in Plate XXXVIII, Fig. 22. The smaller cells show about the same extent of chromatolysis as in Rabbit No. 2.

RABBITS, Nos. 5 AND 6.—*Spinal Cord.* There is less congestion than in any of the preceding cases. There is no change in the small round-cell infiltration of the walls of the blood-vessels.

Cells of the anterior horn. All of the previously described types of degeneration are found. It is still possible to find normal cells, though they are rare; none were found except in the extreme ventral and lateral region of the horn. Of altered cells, few show the earlier changes with normal or slightly changed nuclei and with simply frayed-out chromophilic bodies. More extreme degrees of change, as shown in Plate XXXVI, Fig. 13, are the more common.

Nucleus and contents. The nucleolus, in addition to the changes already described, may be completely disintegrated and represented only by a few fine or coarse blue granules scattered throughout the nucleus.

The nuclear reticulum has, in the majority of cells, disappeared, the entire nucleus taking a granular or diffuse pink stain.

The nuclear membrane is often extremely distorted and broken, sometimes absent. In cells showing more marked forms of degeneration, there may be no trace of nucleus or nuclear contents found, this being determined of course by serial sections. Such apparent absence of the nucleus is most common in cells in which the protoplasm takes a dark stain; while in those cells in which there is a lighter stain, remnants of the nucleus are always found. It would seem probable that the disintegrated nuclear elements, basophile and acidophile, are simply indistinguishable among the similar elements of the cell body proper, when both are in a fine state of disintegration. Eccentricity of the nucleus, while found, is not a common occurrence.

Cell body. Of the conditions already described, the most common is that in which the basophile elements are arranged in the form of a reticulum upon the acidophile network, as described in Rabbit No. 1, type 3 (Plate XXXVI, Fig. 11). In most of these cells, however, the network is much less regular. We may note also the following:

1. Cells in which there is apparently complete disintegration of all stainable elements, both acid and basic. These cells take on a somewhat different appearance according to the greater intensity of the acid or basic stain. With erythrosin alone they take a strong, homogeneous, or granular red stain, with or without scattered, larger, brighter, red granules. With methylene blue alone, they present the

same picture, substituting the blue for the red. With a double stain, they may present a homogeneous appearance, usually of a violet color, the tint inclining towards the red or towards the blue, according to the overstaining. The majority of such cells, however, are not homogeneous, but granular, in which case it is always possible with a proper staining to differentiate red granules from blue granules; the one evidently representing the disintegrated remains of the acidophile reticulum, the other, of the basophilic elements of the cell. In most such cells, the protoplasmic processes are shrunken and irregular in outline. In many, some or all of the dendrites are traceable only a short distance from the cell body, ending usually in points, or in little shrivelled strings. These shrunken, broken processes stain considerably more intensely with erythrosin, than does the cell body to which they belong. They may show a few small remnants of the chromatic spindles; more commonly, however, they present nothing but a homogeneous or granular red appearance (Plate XXXVI, Fig. 11, and Plate XXXVI, Fig. 13, a). There is no evidence of any reticulum. Such cells are usually normal in size, or swollen. Their outline may be distinct and smooth, very indistinct, or, less commonly, broken and ragged.

2. A very common condition of the cell body, more common than that just described, corresponds partly to that noted in Rabbit No. 1, type 3. The basophile element is not greatly reduced. In parts of the cell, commonly the peripheral, it is present as a network though much broken up (Plate XXXVI, Fig. 11); in other parts, it is distributed as irregular, granular masses, apparently situated on nodal points of the acidophile reticulum, the larger covering several meshes. In addition are present, often in clumps, long slender blue rods. These are very narrow, straight or wavy, stained intensely, and appear to have no relation to the acidophile network (Plate XXXVI, Fig. 13, b). They give to the cell a quite characteristic appearance. In such cells the acid reticulum usually remains intact, at least in a portion of the cell. The cell body is apt to be shrunken. The dendrites, if shrunken at all, are only slightly so, and, as a rule, contain chromophilic bodies, somewhat broken up. The cell outline is often

ragged and indistinct. Such cells are most common in the periphery of the anterior horn, and represent the larger cells.

Spinal ganglia. Cells of the posterior root ganglia at all levels of the cord, show changes, with no differences in their extent for different levels. The *nucleus* may be in any of the conditions already described in the case of cells from other regions. In those cells in which there is distinct central chromatolysis, the clear area, just outside the nuclear membrane, brings the nucleus out in strong relief. In other cells the outline of the nucleus is broken completely or lost. An extremely common condition is a crenated nucleus somewhat shrunken, taking a diffuse granular pink stain, containing an indistinct rough-edged nucleolus. Eccentricity, sometimes so marked as to cause bulging of the cell periphery, is not uncommon (Plate XXXVII, Fig. 16, e).

Cell body. 1. There are cells which in the nature of the change resemble the anterior horn cells, type 1, Rabbit No. 1 (p. 585). They show the normal number, size and arrangement of chromophilic bodies, but these have frayed-out, feathery edges.

2. A very common condition in these cells is central chromatolysis (Plate XXXVII, Fig. 16, d, e). The central portion of the cell may be pale blue, with or without very fine blue granules scattered through it, or perfectly colorless. The extent of the chromatolysis varies from a small area immediately around the nucleus, to an involvement of most of the cell, leaving but a narrow rim of chromophilic bodies at the extreme periphery. In the latter case, these bodies are usually large, very dark, run into one another, lie parallel to the outline of the cell body, as if they were being pushed out by centrifugal pressure (Plate XXXVII, Fig. 16, b).

3. A peculiar condition noted in a few cells, consists in a perfectly clear background with a continuous or a broken cytoreticulum, and, scattered quite evenly through it, rather small chromophilic bodies, which are smooth in outline and seem perfectly homogeneous (Plate XXXVII, Fig. 16, c).

The cells of Purkinje show a marked progression of the lesion as compared with the preceding specimens. They are diminished in

number. In cells arranged in distinct rows, Purkinje's cells and the mitral cells from the olfactory lobe, there is a fairly even frequency for a given thickness of section. Thus comparing a 6 μ section of the present specimen with a 6 μ section from a normal rabbit, a marked loss of cells is easily recognized. Few cells are normal. Many are found which present the picture described for the Purkinje cells of Rabbits Nos. 3 and 4. A majority, however, are in more advanced conditions of degeneration, as shown in Plate XXXVII, Fig. 17.

In these degenerated cells the nucleus may present a great variety of appearances. It may be irregular in outline. The intra-nuclear network may be normal, or it may show in a part of the nucleus, while in the remainder it is represented only by reddish granules. The nucleus may have no distinct outline, but appear simply as an area of pale pink granulations (Plate XXXVII, Fig. 17, c). It may be a shrunken red mass, as in Plate XXXVII, Fig. 17, d. In most of the nuclei there was a coarse blue granulation (Plate XXXVII, Fig. 17, b, c, d). In some, this appeared to be due to a disintegration of the nucleolus; in others, it seemed equally extensive without any reduction in the size of the nucleolus.

In the bodies of most of the cells there is extremely little chromatic substance. When present it is as short threads or rods, or as small granular masses. The reticulum in these cells is indistinct, showing, if at all, only at the periphery of the cell (Plate XXXVII, Fig. 17, a). The remainder of the cell body often has a granular pink appearance. The outline of the cell is in many cases irregular and the processes are indistinct.

Cerebral cortex (Plate XXXVII, Fig. 18). The changes in the cortical cells show a considerable advance, as may be seen by comparison of Figs. 18 and 23. It should be noted that only the drawing of a very large number of cells could give any adequate picture of the shades of variation which these cells present. Many cells still remain normal; these, however, are much less frequent than in the cortices of the preceding rabbits, and more of the changed cells show the more extensive modifications. Plate XXXVII, Fig. 18, b, shows one of the less affected cells, which resembles quite closely those seen

in Fig. 23. The nucleus is distorted. In cell, a, of the same figure there is considerable shrinkage of the cell body and but little evidence of the reticular arrangement of the granules. The nucleus is irregular, eccentric and only a few shreds of its reticulum remain. In c of the same figure the loss of basophile substance is complete, leaving an extremely clear demonstration of the apparently unchanged acidophile reticulum. As in rabbits 3 and 4 there is no special localization of the cerebral lesion, the cells being affected to an equal extent in all regions.

Mitral cells of the olfactory lobe (Plate XXXVII, Fig. 19). Even more marked than in the cells of Purkinje is the progress which the lesion has made in the mitral cells. As in the cerebellum, there is a decided and even a greater reduction in the number of cells. One can in places pass along the line of mitral cells for several fields of a No. 7 lens without seeing a single mitral cell. The least changed cells show the outlines of some of the larger chromophilic bodies. In cell a of Fig. 19 most of the large chromatic masses are broken up and represented by rather small collections of granules; where the larger chromophilic bodies remain, they are ragged and fenestrated. The cytoreticulum in cells which are of about normal size, is usually quite distinct. In Fig. 19, are seen two of the least changed cells from the olfactory lobe of Rabies Rabbit No. 5. They are in many respects similar to those in Plate XXXVI, Fig. 12 of Rabies Rabbit No. 3. The disintegration of the chromophilic bodies is more extensive and the actual amount of basophile substance is less. In the dendrites the basophile granules are usually arranged as fine straight, or more often wavy lines and as oval masses in the varicosities; in this way they often project somewhat from the surface like knots on a branch of a tree, giving the process a rough, wavy appearance. In these cells the nucleus is apt to be distorted and the nucleo-reticulum more or less disintegrated.

Smaller cells are found, which are shrivelled and irregular in outline, and with little appearance of structure remaining. The nucleus is represented usually by a shrunken red or purple mass around the remains of the nucleolus. Broken parts of cells, pieces of cell

bodies and of dendrites, are seen scattered along the line occupied by the mitral cells.

Basal ganglia. The large cells of the corpus striatum and of the thalamus present much the same appearance as the cells of the anterior horn (Plate XXXVI, Fig. 13). A considerable number of cells show a rather extreme condition of chromatolysis giving them the appearance depicted in Plate XXXVIII, Fig. 22. The network arrangement of the granules is not so frequent as in the cells of the cord. Vacuoles of various sizes in the cell body seem to be of rather more frequent occurrence than in the anterior horn cells. The loss of basophile substance in the smaller cells has become more pronounced. Few of the cells show any of the large blue masses; most show only a fine blue granulation, some an almost complete chromatolysis.

RABBITS Nos. 7, 8, AND 9.—These rabbits, one dying on the eighth day and the others on the ninth day after inoculation, showed such similarity in the lesions, that they will be discussed together, any individual peculiarities being noted.

Spinal Cord and Medulla.—Congestion is present and quite intense in Rabbit No. 8, very slight in Nos. 7 and 9. It is uniform throughout cord and medulla.

Hæmorrhages, mostly in the grey matter, and at different levels of the cords, are frequent in all three rabbits. They occur (a) into the grey matter without definite limitations; (b) into the peri-vascular lymph spaces, and (c) into the peri-cellular lymph spaces around nerve cells, as shown in Plate XXXVII, Figs. 20 and 21, the nerve cell being pressed by the blood to one side of the space. Plate XXXVII, Fig. 21, shows a blood-vessel and its relation to the cell space, and peri-cellular hæmorrhage. In several instances the plane of the section was such as to make a peri-cellular hæmorrhage appear as if situated inside the cell itself, entirely surrounded by cell substance.

Changes in the walls of the blood-vessels are not nearly so pronounced as in some of the rabbits killed earlier in the series. Only a few vessels, mainly in the medulla, show slight infiltration of their walls with leucocytes. The increase in connective tissue elements is also not common, and usually only a few new cells are present.

Cells of the anterior horn (Plate XXXVIII, Fig. 24, and Plate XXXVI, Fig. 14). No normal cells were observed. With the exception of the very earliest stage, in which there is little else than a ragged or somewhat ragged appearance of the outlines of the chromophilic bodies, all of the types of degenerative condition already described are present. The great majority of the cells, however, fall into one of the two following classes:

1. Cells in one respect similar to those already described in which there is complete or almost complete chromatolysis. They show certain further changes which appear to mark the progress of the degenerative process.

(a). The *nucleus* is usually irregular in shape; its membrane may be distinct, thickened, and stained a brilliant red. Within this nucleus there is no distinct reticulum, but a larger or smaller number of round or irregular, apparently homogeneous masses, staining bright red, and most numerous around the nucleolus. Some of these masses are often as large as the nucleolus itself. The apparent total amount of this acid-staining nuclear substance, is often much greater than in the normal cell, and is difficult to explain in any other way than by supposing an absolute increase in the acidophile elements of the nucleus. Instead of these distinct red globules, the entire nucleus may be simply an irregular bright red mass, with or without a nucleolus, and with or without a visible enveloping membrane. There is sometimes no nuclear limitation, a nucleolus surrounded by a few bright red granules, or the red granules without the nucleolus, lying loose in the general cell protoplasm. Less commonly there is entire disappearance of the nucleus.

(b). The *cell body* shows no evidence of chromophilic bodies or of a cytoreticulum. The acidophile portion of the cell body is represented by a homogeneous, or finely granular pink or bright red background. There is considerable difference in the intensity of the stain in different cells and in different parts of the same cell. Speaking generally, the stain is lightest in the central portion of the cell, and grows darker as the processes are approached. The processes themselves are much darker than the cell body proper, this being especi-

ally true of the axis cylinder process and its axone hillock, which often stands out with an extremely bright red stain. The protoplasmic processes have, like the cell body, lost all trace of a reticular appearance, but often show an indefinite longitudinal striation. There may be no signs of the blue-staining element of the cell; or there may be some coarse blue granules standing out sharply against the red background; or there may be a very fine blue granulation, giving the cell a diffuse violet or purple color (Plate XXXVIII, Fig. 24). The same thing impresses one in regard to the acid elements of the cell body, as has already been noted in regard to the acid elements of the nucleus, namely, a very decided increase, or at least an apparent increase in these elements. When we remember how very delicate and lightly staining are the strands of the cytoreticulum in the normal cell, it seems necessary to think of either an absolute increase in the amount of acidophile substance, or else that, occurring in a different condition, as diffuse granules or dissolved in the general cell protoplasm, it so takes up the red stain as to appear present in greater quantity. Such cells are usually not shrunken, but entirely or almost entirely fill up the cell spaces. Their dendritic processes are reduced in diameter, and often taper rapidly to a point but a short distance from the cell body. They contain little or no chromatic substance.

2. Cells in which there is a considerable amount of chromatic substance, and this substance deposited in quite a characteristic manner.

(a). The *nucleus* in these cells is often perfectly round, with a broad, bright red nuclear membrane and is usually in the centre of the cell. There is no distinct intra-nuclear network. The most common condition within the nucleus is that already described—an apparent increase in the acidophile element, which is present as round or irregular masses of considerable size. More rarely the nucleus is crenated and may be in any of the conditions previously described.

(b). In the *cell body* an imperfect cytoreticulum is often visible in some parts, the remainder of the cell showing a diffuse pink background, homogeneous, or more often finely granular. As in the previous type of cell, the processes show a stronger red than the cell body, but in this type they usually contain some remnants of the

chromatic spindles. Moreover, they do not taper off at so short distances from the cell as do those of the preceding type.

It is in the chromatic element that these cells show their peculiarity. It will be remembered that in Rabbits Nos. 5 and 6 (Plate XXXVI, Fig. 13, b), was noted the occurrence of a part of the chromatic substance as dense thin rods, straight or wavy, and taking a very dark homogeneous stain. Here there has been both a decided increase in the number of cells in which the chromatic substance is deposited in this manner, and an increase in the proportion of chromatic elements thus deposited. The remaining chromatic substance is present as irregular masses, some small, some showing the general shapes of large normal Nissl bodies, but full of vacuoles and with edges which, instead of being granular, appear hard and sharp cut. These edges and the edges of the vacuoles take a very dark homogeneous stain. Among these are found a few chromophilic bodies of the usual granular types, taking by contrast a rather pale blue stain. It is impossible to tell whether there is in such cells a loss of chromatic substance, and if a loss, how extensive. It would appear rather as if there was a contraction or shrinkage of the more loosely aggregated finely granular chromophilic bodies into the hard dense masses seen in these cells.

Spinal Ganglia (Plate XXXVI, Fig. 15). The changes resemble those already described in Rabbits Nos. 5 and 6. More cells are affected, there being none, apparently, that are normal.

The nucleus is usually more or less shrunken or crenated and is often eccentric. It may correspond to the nuclear condition just described in anterior horn cells, in which there is an apparent increase in acidophile elements, these being present as larger or smaller discrete masses or even distributed so as to give a diffuse red stain to the entire nucleus. The nucleolus is in most cases round and smooth and much the same as in the normal cell.

The basophile element of the cell body is somewhat more reduced than in the preceding cases, though not markedly. That condition described as type 3 in the ganglion cells of Rabbits Nos. 5 and 6 (p. 597), is more frequent. These cells in which the bright blue bodies are small, sharp-edged, smooth, quite homogeneous, and evenly

distributed through the clear or pink cell body, present quite a characteristic picture.

The cytoreticulum is in most cases entirely indistinguishable. When visible, it is usually around the periphery of the cell, and even then much disintegrated. The general cell body takes a diffuse stain, varying from a light pink to a pronounced red. It is homogeneous, or, more rarely, finely granular. Vacuoles are frequent, often very large, one sometimes occupying a quarter of the cell body. Distinct disintegration of the cell body is not uncommon, when all that remains of the cell is a shrivelled mass of pinkish staining substance, with or without a few small blue masses, hung by thread-like strands to the edges of the cell space (Plate XXXVI, Fig. 15, b).

Cells of Purkinje. As compared with Rabbits Nos. 5 and 6, there seems to be no further diminution in number of these cells, nor do they show any further loss of chromatic substance. The nucleus is oftener irregular and crenated, but as a rule it presents a distinct, coarsely granular network and membrane, and a normal nucleolus. It is often eccentric, sometimes lying on the edge of the cell. The outlines of the cell body have also become more irregular, often shrunken. There is a distinct reticulum. Stained with erythrosin alone, this presents a granular red appearance; with methylene blue alone, a granular blue; with the double stain, a granular violet or purple. Around the periphery of the cell, it was possible to make out, in double stained specimens, a few strands of the reticulum, stained red and uncovered by blue granules; it seems probable, therefore, that in the central areas, the same condition obtains as in the stichochromes of the anterior horn, namely, an acidophile reticulum incrustated with fine basophile granules. In these cells, however, a satisfactory demonstration of this could not be secured.

The main protoplasmic process and its branches are apt to be wavy and knobbed. They contain much of the basophile substance in the form of fine granules, often strung along in rows. The cone of bifurcation of the first division of the main dendrite often preserves its general outline, while having a ragged eaten-out appearance.

Cerebral cortex. The cerebral congestion was not marked in any of these three rabbits, nor were the vascular changes at all extensive.

As was the case with the cells already described from the anterior horn, medulla, spinal ganglia and cerebellum, there was little apparent progress in chromatolysis of the cortical nerve cells. There are many normal cells, that is, many which contain a relatively small amount of chromatin, arranged as a network and without much thickening at the nodal points. There are few, however, of those cells found in the normal cortex, which are rich in chromatin, having large nodal masses and chromophilic bodies which cover over several of the meshes of the reticulum. It seems probable, therefore, that many of the apparently normal cells represent cells which normally would have a larger amount of chromatin, and in which the chromatolytic change has resulted in loss of the larger chromophilic bodies, the chromatic network remaining. The probability of the correctness of this deduction is enhanced by the fact that some of these cells show in ragged outline skeletons, as it were, of once larger chromatic masses, and that these cells are prone to show more or less advanced nuclear changes and irregularity of dendritic processes. Degenerative progress is shown, however, in these cells, as in the cells already described from other parts of the nervous system of these same rabbits, by changes in the nucleus and in the outlines of the cell body and processes.

While in most cells the nuclear membrane appears with increased distinctness, its outline is irregular, this irregularity varying from a slight depression or projection at one point to marked crenation of the entire nucleus. There is usually more or less diffuse staining of the nucleus, its depth depending largely upon the amount of shrinkage, being slight in nuclei of normal size, and extreme in those with marked shrivelling and crenation. The condition of the nuclear reticulum varies, its clearness, of course, depending largely upon the extent of the diffuse staining. It is often seen to be quite disintegrated, however, in nuclei which show no general staining. The nucleolus is usually ragged and irregular, sometimes broken up into several pieces.

The outline of the cell body shows, in most cases, some irregularity; it may be merely a waviness of contour, or less frequently a decided shrinkage of the cell body around the nucleus. Between

these extremes are all gradations. There is usually a fine line of chromatic granules around the periphery, emphasizing the raggedness of the cell contour. The same irregularity extends out into the dendrites, often more marked there than in the cell body proper. The varicosities are often quite large, and between them the process may be reduced to a mere thread. A finely granular chromatic substance is abundant in the varicosities. The diameter of a process through one of these varicosities is usually not greater than that of the normal dendrite, while between them the diameter is greatly reduced. It would thus seem that the varicose condition is due mainly to shrinkage between, rather than swelling at, the points of the varicosity, and that the varicosities themselves depend upon clumps of basophile granules. Plate XXXVIII, Fig. 25, a, shows one of these more changed cells, and in b of the same figure is seen an atrophied dendrite containing basophile granules. The staining is by methylene blue alone.

Mitral cells of the olfactory lobe. The description already given for the mitral cells in Rabbits Nos. 5 and 6, applies equally to those in Rabbits Nos. 7, 8, and 9, as regards the general character of the changes. There has been a further decrease, however, in the number of cells, and more of those remaining show the more intense grades of change. No normal cells are present, and few show any large chromatic masses. Fine granules strung out in ragged wavy lines represent the remnants of the Nissl bodies. Sometimes in rough outline are seen the skeletons of the larger Nissl bodies, described in other cells. There is little if any further loss in chromatic substance. In many even of the most shrunken cells the main dendrites appear of nearly normal diameter, though extremely wavy. In these are seen the fine, rough, broken lines of blue granules lying upon the strands of an only slightly disintegrated acidophile reticulum. Other more shrunken dendrites are varicose, devoid of reticulum, stain diffusely dark red, and show an arrangement of chromatic substance like that described in the cortical cells similarly affected. Raggedness of outline of cell body and of nucleus, with shrinkage of both, is the rule. No more striking picture is seen in any part of the central nervous system of these rabbits, than that presented by this

row of mitral cells. In the normal olfactory lobe these cells present a very uniform appearance and even distribution along the edges of the granular layer; the bulk of their chromatin is in large blocks, is very dense and stains intensely. When one examines, even with a low magnification, this line of mitral cells in any of the later rabbits of the series, the changes are seen to be extremely pronounced. The long stretches entirely free from nerve cells, the ragged, shrunken bodies of most of those remaining, with their shrivelled and eccentric nuclei and disintegrated chromatic bodies, the many long, irregular, wavy parts of dendrites, scattered along even where there are no cell bodies, present altogether a very striking picture.

Basal ganglia. The large cells here show about the same grade of lesion as do the stichochromes of the ventral horn, the description of the latter applying equally to the former. There are almost no normal cells. Most of the cells are in a condition of well advanced degeneration; in many there is extreme chromatolysis, even more marked than that depicted in Plate XXXVIII, Fig. 22. The types pictured in Plate XXXVI, Fig. 13, are frequent, the same hard wavy lines seen in cell b of this figure being present. Nuclear changes are identical with those already described in the ventral horn cells. Most of the small cells show almost complete chromatolysis, with distortion of the cell bodies, loss of processes and more or less complete disintegration.

I desire to express my thanks to Dr. Follen Cabot and to Mr. James P. Atkinson of the Research Laboratory of the Department of Health of the City of New York, for material placed at my disposal and for their courtesy in the inoculation of a series of rabbits with the virus of rabies.

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DESCRIPTION OF PLATES XXXIV-XXXVIII.

Unless otherwise noted, drawings were made with a Reichert $\frac{1}{12}$ homog. imm., apert. 1.25, from specimens 6μ thick, stained with erythrosin-methylene-blue.

THE NORMAL NERVE CELLS.

PLATE XXXIV.

Fig. 1. Typical spinal stichochrome from ventral horn of cord of a normal rabbit. The acidophile reticulum is seen in the axone hillock extending out as delicate longitudinal striations into the axis cylinder. In the protoplasmic processes the reticular meshes are elongated.

Fig. 2. Drawing made with a Reichert $\frac{1}{18}$ homog. imm., apert. 1.30, sections 3μ thick, representing portions of ventral-horn stichochromes.

a. A protoplasmic process in longitudinal section, showing elongated meshes of the acidophile reticulum and the longitudinally arranged chromophilic spindles lying upon them.

b. Cross section of a protoplasmic process showing acidophile reticulum and cross-cut chromatic spindles lying on and around the strands of the reticulum.

c. Longitudinal section of an axone, showing striations and their relations to the intra-cellular reticulum.

Fig. 3. Drawings made with a Reichert $\frac{1}{18}$ homog. imm., apert. 1.30, sections 3μ thick, representing portions of ventral-horn stichochromes. Shows relation of basophile granules to acidophile reticulum. Granules are seen to lie upon meshes of reticulum.

Fig. 4. From a spinal ganglion, lumbar region; section 6μ thick. Shows types of cells found in spinal ganglia with the variations in size of cells, in size of nuclei, and in the size and arrangement of the chromophilic granules. In two of the cells (a and a'), one large and one small, the chromophilic granules are arranged as short rods curved slightly so as to present a concavity towards the nucleus and giving a concentric appearance to their arrangement. Another cell (b) shows the fine granular condition of the chromatic substance with an almost even distribution throughout the cell-body, there being a few larger chromatic masses present. The fourth cell (c) shows a rather coarse reticular arrangement of the granules, the reticulum corresponding somewhat to, though being coarser meshed than, the acidophile reticulum.

Fig. 5. Typical Purkinje cells from the rabbit's cerebellum. In one of the cells, a, the plane of section includes a large part of the main dendrite with primary and secondary bifurcations and gives a clear picture of the dendritic reticulum and the

overlying rods of granules. The nucleus is rather small as compared with the cell-body, and its reticular strands are coarse. In all of the cells drawn the "nuclear cap" is distinct, as is also a tendency, not nearly so marked as in the cells of the spinal ganglia, to a concentric arrangement of the granular masses.

PLATE XXXV.

Fig. 6. Represents two cells from the mitral layer of the olfactory lobe. The chromophilic bodies are extremely coarse, and there is a well marked nuclear cap. The rods in the dendrites are large. The chromophilic masses take a very dark stain. The acidophile reticulum is of comparatively fine mesh, and does not show well any distinct relation to the chromophilic granules.

Fig. 7. Four cells from the frontal lobe of the cerebrum. One of these (a) is cut at right angles to the direction of the main dendrite, and is from a section tangential to the surface. The others are cut in the axis of the main dendrite and are from transverse sections cut at right angle to the surface of the brain.

Fig. 8. Three cells from the cerebrum. The section was a perpendicular one, and taken at a point midway between the tip of the frontal lobe and the tip of the occipital. The cells average considerably larger and are richer in chromatic substance than in the frontal lobe.

Fig. 9. Five cells from the occipital lobe. These again are smaller than those found in the mid-region, and the chromatic constituent is less.

RABIES.

PLATE XXXVI.

Fig. 10. From ventral-horn cells, lumbar cord, Rabies Rabbit No. 1. Sections 3μ thick; Reichert $\frac{1}{8}$ homog. imm., apert. 1.30. Early changes in the chromophilic bodies and relation of these to the acidophile reticulum.

a. Frayed out appearance of edges of chromophilic bodies without any diminution in staining intensity.

b. Frayed edges with considerable diminution in staining intensity.

c. Same as b, but with holes or vacuoles in the chromophilic bodies. These holes seem to correspond to the meshes of the reticulum.

Fig. 11. Cell of ventral horn, cervical cord, Rabies Rabbit No. 3. One of the cells showing most marked changes found in this rabbit. Extreme eccentricity of the nucleus, which bulges somewhat from the cell-body; loss of any distinct nuclear contour; no nuclear membrane; no intra-nuclear network; nucleolus broken up into three distinct parts; acidophile element of nucleus forming a stellate granular mass around the nucleolus; some diffuse red staining of the rest of the nucleus. Cell processes short, tapering to a point near the body of the cell; some appear like mere reddish threads. Only one of the processes shows any evidence of either reticulum or chromophilic rods; the other processes show an increased intensity of reaction to erythrosin, but no reticulum. In the cell-body the reticulum is largely obscured. Instead of the usual separate chromophilic bodies there is around the nucleus a diffuse dark-blue granular deposit, while towards the periphery the chromatic granules are arranged in a reticulum, the meshes corresponding to some extent to the remains of the acidophile reticulum.

Fig. 12. Two large mitral cells from the olfactory lobe, Rabies Rabbit No. 3.

In a, all the large chromatic masses have disappeared, what is left of the basophile element being scattered throughout the cell as small clumps of granules, in irregular rods, and in one end of the cell as a distinct reticulum. The nucleus shows a partial disintegration of its reticulum and the stellate collection of red granules around the nucleolus.

In b, some large chromophilic bodies still remain, ragged and frayed out at the edges. In some parts of the cell there is a diffuse granular deposit; in other parts there is a distinctly reticular arrangement of the granules, which lie on the strands of the acidophile net work. The nucleus is distorted, as is also the nucleolus. The changes in the nucleo-reticulum are the same as in a.

Fig. 13. Ventral-horn cells from cervical cord, Rabies Rabbit No. 6.

a. Nucleus crenated, nucleolus broken up, and nucleo-reticulum almost completely disintegrated. In the cell-body the basophile element is represented only by a few scattered granules. The body of the cell stains a diffuse and rather dark red. The processes end in points at a short distance from the cell-body, these points taking a darker red stain than any other portion.

b. Shows disintegration of larger chromatic masses; outlines of some still show; wavy rods of chromatic substance which have a very hard appearance and take an intense dark-blue stain; cytoreticulum normal throughout greater portion of cell; vacuoles in several places, where both reticulum and chromatic substance are wanting.

Fig. 14. Two cells from ventral horn, lumbar cord, Rabies Rabbit No. 9, transverse section.

a. Cell-body shrunken to one side of cell space, leaving strands of cell substance stretching across between the cell and the edges of the space, and presenting the appearance of large vacuoles. No very distinct chromophilic bodies, but the basophile substance present in rather large amount, deposited as more or less closely packed granules, in some places making a coarse reticulum. A single process is seen which takes a dark-red stain.

b. Resembles preceding, except that the destruction has gone farther. At one side is seen a short, pointed protoplasmic process, entirely disconnected with the remains of the cell-body.

Fig. 15. Three cells from a cervical posterior root ganglion, Rabies Rabbit No. 9.

a. Cell-body shrunken somewhat from cell space, leaving a net-work appearance between the cell-body and the edges of the space; diffuse dull-pink staining of cell-body with rather large dark-blue granules scattered through it; nucleus distorted; nuclear reticulum broken; one stellate red mass, about the size of nucleolus, in the nucleus.

b. Cell-body shrunken to dull-pink mass about centre of space leaving strands, and an irregularly shaped band of cell substance crossing between the central mass and edges of space.

c. Cell fills space excepting at one side where there is a large vacuole; nucleus irregular and takes a homogeneous dark-red stain; reticular arrangement of chromatic substance near periphery of cell, and considerable diffuse blue granulation.

PLATE XXXVII.

Fig. 16. Portion of a cervical spinal ganglion, Rabies Rabbit No. 6, showing five nerve cells.

a. Only a remnant of nucleus and nucleolus; acidophile reticulum apparently intact; basophile element of cell-body entirely gone.

b. Nucleus nearly normal. Almost no basophile substance in the central portion of the cell; irregular line of chromophilic bodies around the periphery; acidophile reticulum normal.

c. Nucleus normal; cytoreticulum intact; basophile element present as small, dense, intensely dark-blue masses, scattered quite evenly throughout the cell-body; some diffuse granules in one part of the cell.

d. Intra-nuclear network somewhat broken; some chromophilic bodies around the nucleus and at the periphery, acidophile reticulum normal. Near the surface of the cell its strands are covered by the basophile granules.

e. Intra-nuclear network broken; nucleus eccentric; reticulum normal; cell free from basophile element except near nucleus and at extreme periphery.

Fig. 17. Four cells of Purkinje, Rabies Rabbit No. 6.

a. Eccentricity of nucleus; cytoreticulum normal in part of the cell; diffuse red granules in central portion; reticulum broken in other portions of the cell; basophile granules few and situated near the nucleus and at the periphery on the strands of the reticulum.

b. Nucleus distorted and filled with fine blue and coarser reddish granules; nucleolus large and irregular; no basophile element in cell-body; acidophile reticulum shows in some parts of the cell, but is mostly lost in a diffuse red granular deposit.

c. Nuclear outline lost; no nucleo-reticulum or cytoreticulum; diffuse red granular stain of both nucleus and cell-body; small dark-blue granules in nucleus; basophile substance arranged as more or less concentric rods around the nucleus as a centre.

d. Nucleus distorted and shrunken, taking a diffuse dark-red stain, with very fine blue granules scattered through it; nucleolus crenated; no cytoreticulum; no basophile substance; diffuse granular red stain.

Fig. 18. Three of the more changed cells from the deeper layers of cerebral cortex, Rabies Rabbit No. 6. Transverse section through mid-region of brain.

a. Outline of cell-body irregular; few chromophilic bodies around nucleus, at periphery and in dendrites; diffuse blue granules; reticulum shows only at periphery and in dendrites; nucleus irregular and eccentric; nucleo-reticulum broken.

b. Similar to a, excepting the distinctly reticular arrangement of the blue-staining element in parts of the cell.

c. Extreme eccentricity of nucleolus; cytoreticulum very distinct; complete absence of basophile substance.

Fig. 19. Two mitral cells from the olfactory lobe of Rabies Rabbit No. 5, transverse median section.

a. Nucleus eccentric but not much changed; outlines of some of the larger chromophilic bodies well preserved, but bodies themselves ragged and full of holes, giving a fenestrated appearance; reticular arrangement of some of the granules; cytoreticulum normal.

b. Similar to a, except that nucleus is central; shows the arrangement of chromophilic bodies in large dendrite; they are placed along the margins of the process and being somewhat wavy, give the process a wavy appearance.

Fig. 20. Ventral-horn cell, cervical cord, Rabies Rabbit No. 7. Haemorrhage into pericellular lymph space, pressing cell over to one side.

Fig. 21. Ventral horn-cell, cervical cord, Rabies Rabbit No. 9. Similar to preceding. Shows a vessel from which haemorrhage has taken place into a pericellular lymph space; cell-body pushed over to one side; irregular, fenestrated, chromophilic bodies packed closely together.

PLATE XXXVIII.

Fig. 22. Ventral-horn cell, cervical cord, Rabies Rabbit No. 1. Cell was situated in the postero-internal part of the horn. Shows an extreme degree of degeneration for this rabbit. Nucleolus irregular in outline and stains irregularly; nucleoreticulum almost completely lost with the exception of a few thick strands of reddish granules; nuclear outline irregular and indistinct; cell outline ragged and processes gone. Basophile element appears as a fine granular deposit throughout the cell, with here and there a few clumps of granules. Faint acidophile reticulum at the periphery, but throughout the greater portion of the cell the reticulum is either lost or covered up by the diffuse blue granular stain.

Fig. 23. Three of the larger pyramidal cells from the deeper part of the cerebral cortex; transverse section midway between tip of frontal and tip of occipital lobes; Rabies Rabbit No. 5. Disintegration of the nucleoli in two of the cells; stellate arrangement of granules around the nucleolus with breaking up of the nucleoreticulum; in one of the cells the nucleus is markedly eccentric. Disappearance of the larger chromatic masses with a decidedly reticular arrangement of the remaining granules.

Fig. 24. Cell from motor nucleus of fifth nerve; transverse section; Rabies Rabbit No. 9.

Cell-body appears swollen; smooth in outline; filled with fine granules, some of which take the blue, others the red stain. At one end of the cell a considerable area entirely free from the blue granules and from which a process, probably the axone, arises. No distinct nuclear outline, the area of the nucleus taking a granular-red stain like the axone hill, and in the centre of the area a nucleolus surrounded by a stellate purple area apparently the remains of the shrivelled nucleus.

Fig. 25. Mitral cells, olfactory lobe, Rabies Rabbit No. 9, transverse median section, methylene-blue stain only.

a. Shrunk cell-body; reticular arrangement of blue granules; irregular shrinkage of processes, the chromophilic network showing in the broader parts, remaining chromophilic granules seem to cause nodules on dendrites; nucleus and nucleolus crenated.

b. Reichert homog. imm. $\frac{1}{18}$, apert. 1.30, shows even more distinctly than preceding the arrangement of basophile granules in atrophied dendrite.



FIG. 1.



FIG. 2.

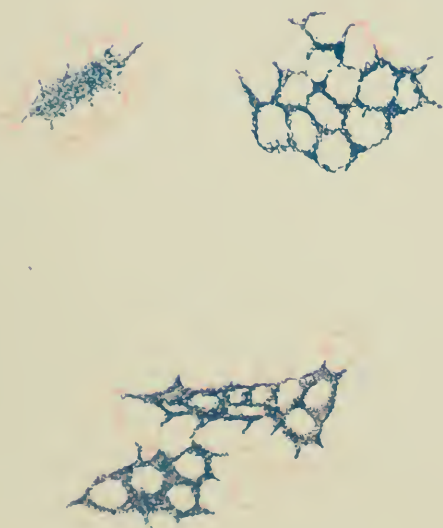


FIG. 3.

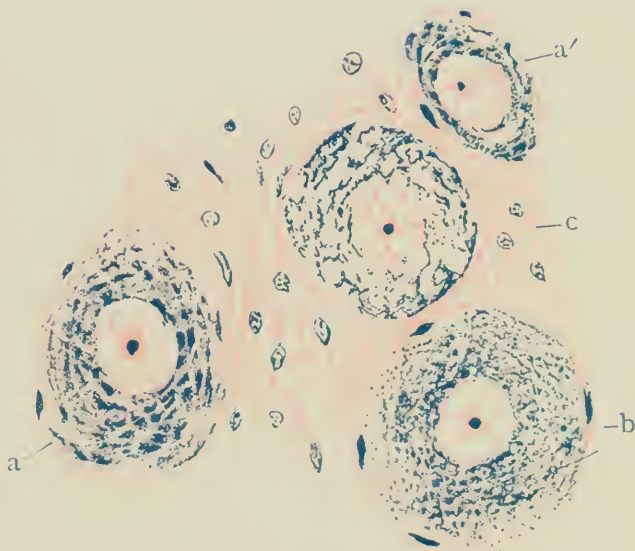


FIG. 4.

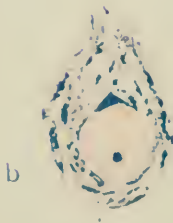


FIG. 5.

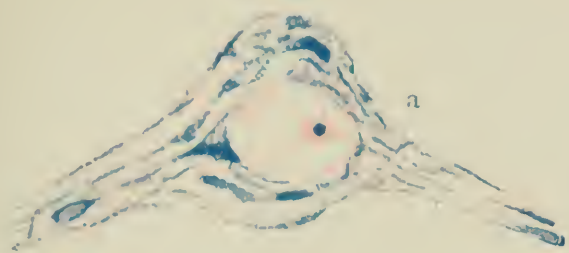


FIG. 6.

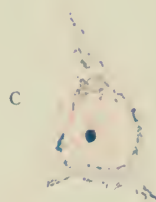
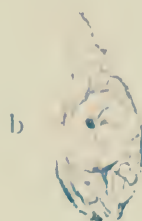
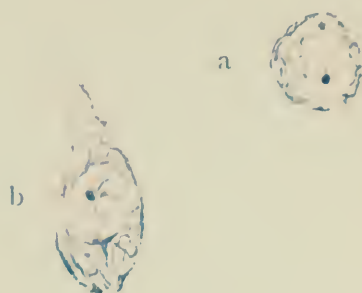


FIG. 7.

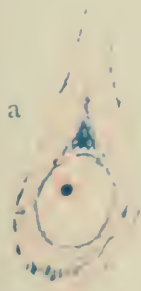


FIG. 8.



FIG. 9.



FIG. 10.



FIG. 13.

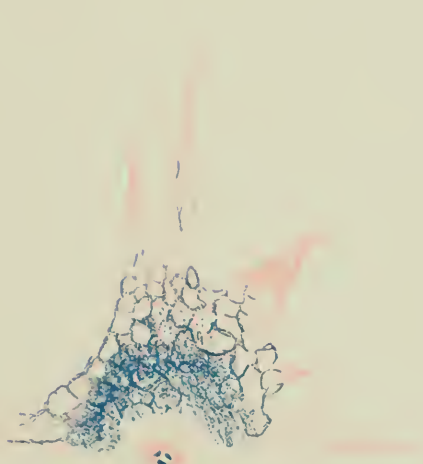


FIG. 11.

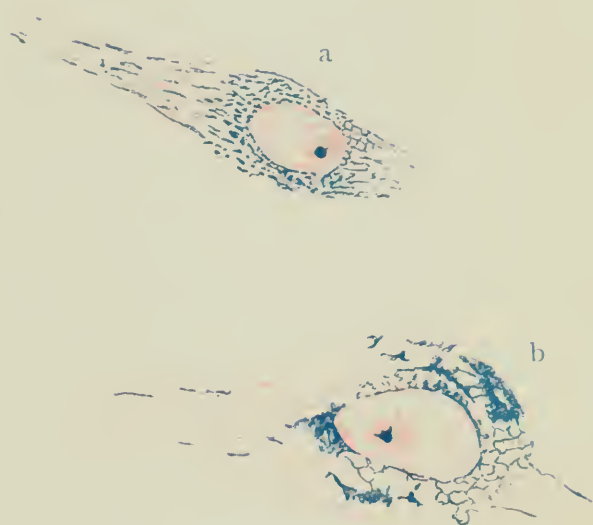


FIG. 12.

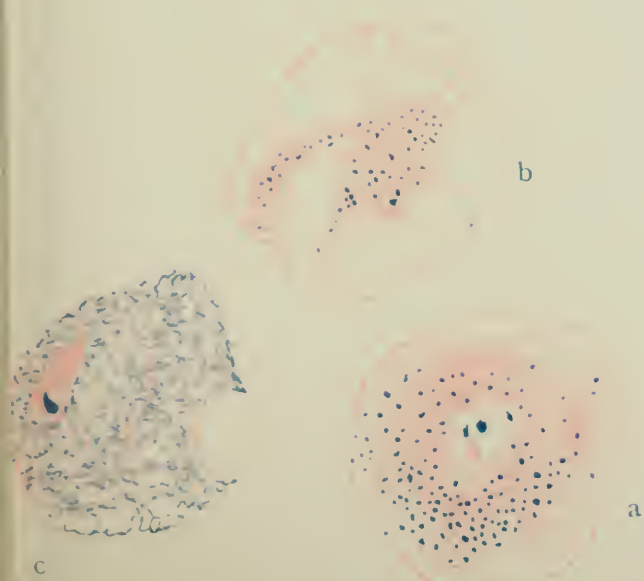


FIG. 15.

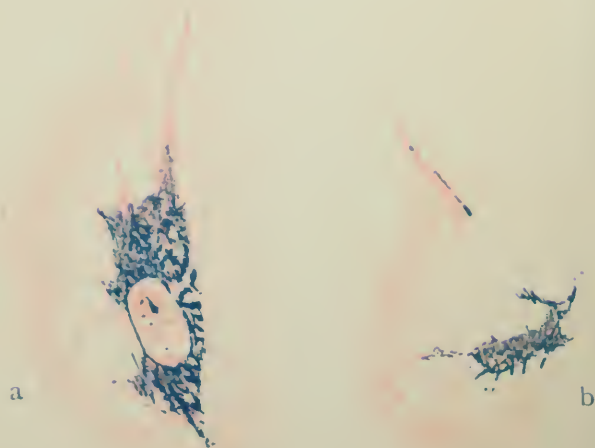


FIG. 14.



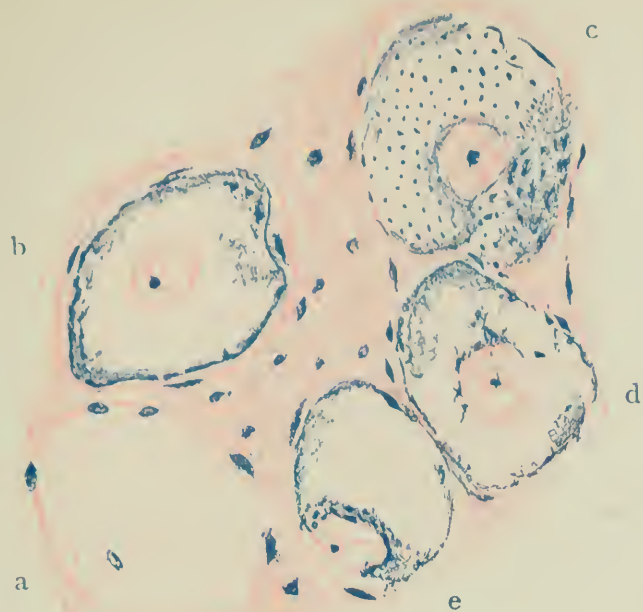


FIG. 16.

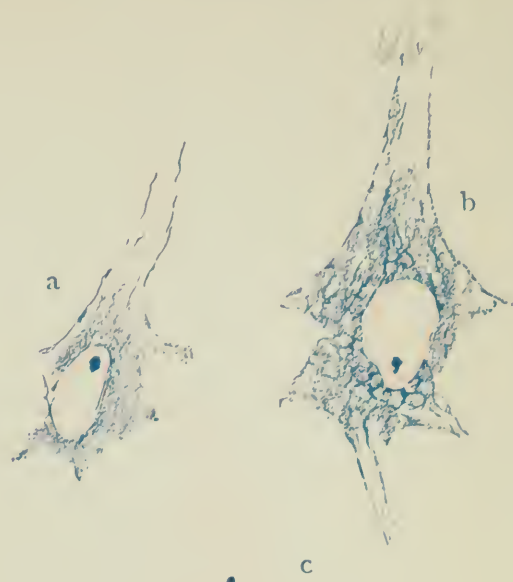


FIG. 18.

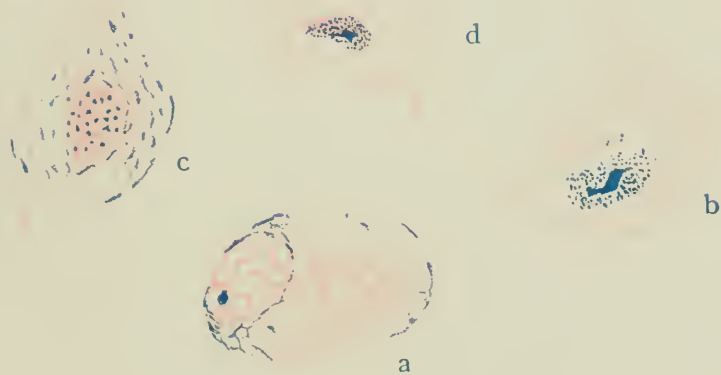


FIG. 17.

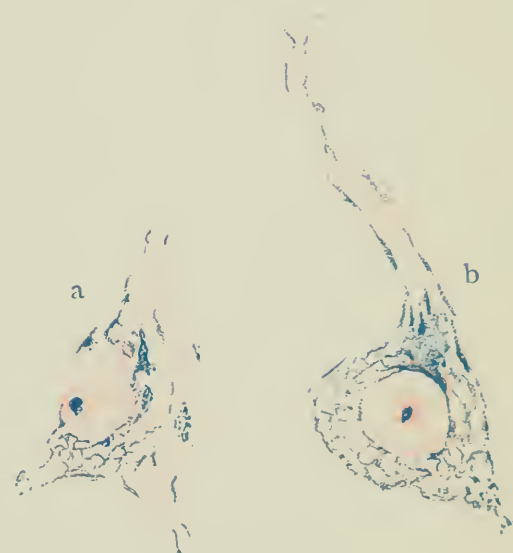


FIG. 19.

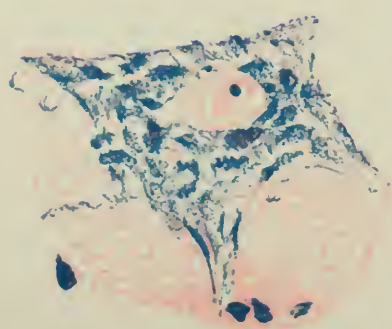


FIG. 20.

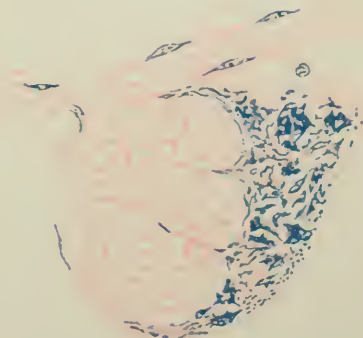


FIG. 21.



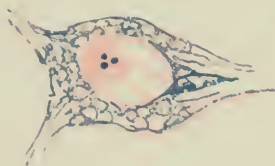
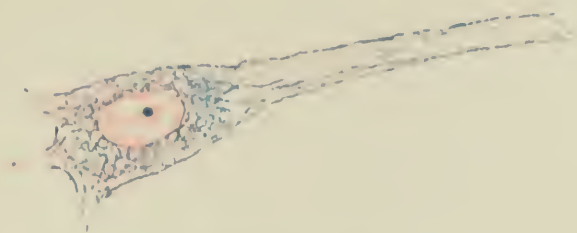


FIG. 23.

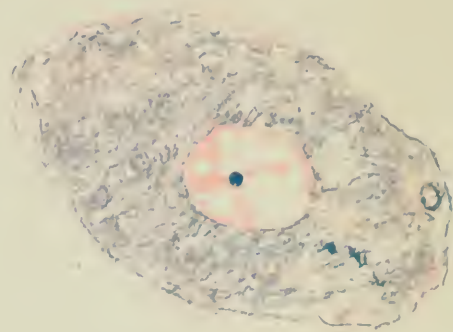


FIG. 22.



FIG. 24.

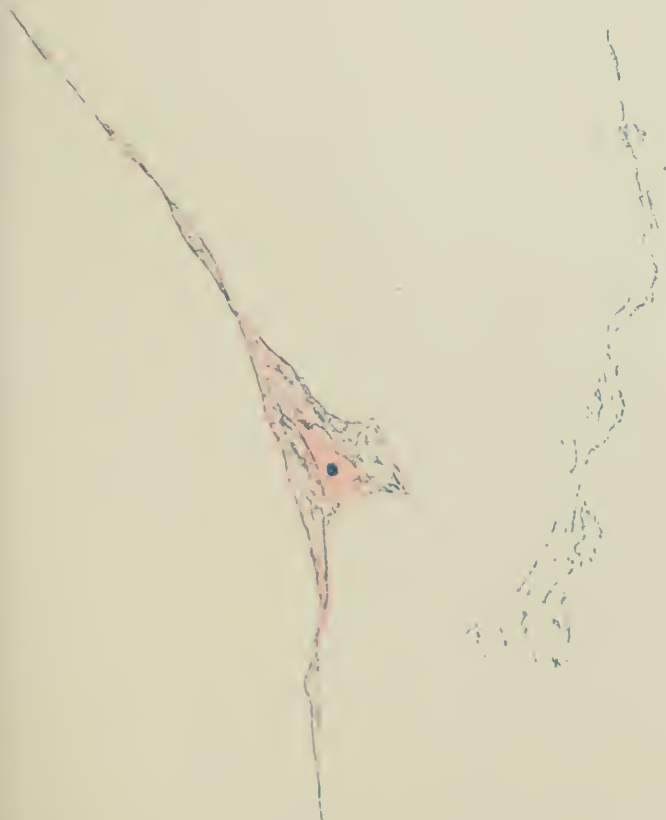


FIG. 25.



THE ACID INTOXICATION OF DIABETES IN ITS RELATION TO PROGNOSIS.¹

BY C. A. HERTER, M. D.

CONTENTS.	PAGE
METHODS OF DETECTING THE PRESENCE OF ACIDS IN THE URINE.....	617
THE EXCRETION OF ORGANIC ACIDS SHORTLY BEFORE OR DURING COMA.....	619
CASES OF DIABETES IN WHICH CONSIDERABLE QUANTITIES OF ORGANIC ACIDS WERE BEING EXCRETED AND WHICH HAVE NOT YET DEVELOPED COMA OR IN WHICH THE OUTCOME IS UNKNOWN	623
CASES OF DIABETES IN WHICH THERE IS EITHER NO PATHOLOGICAL EXCRETION OF ORGANIC ACIDS OR IN WHICH THE EXCRETION IS SMALL.....	627
THE RELATION BETWEEN THE EXCRETION OF ORGANIC ACIDS AND THE EXCRE- TION OF GLUCOSE.....	629
THE NATURE OF THE ORGANIC ACIDS IN THE URINE AND BLOOD OF DIABETES.	630
CONCLUSIONS.....	631

While the admirable studies of Stadelmann,² Naunyn,³ Minkowski⁴ and Magnus-Levy⁵ have firmly established the fact that the peculiar coma so often noted in diabetes is regularly associated with the excretion of large quantities of organic acids, comparatively little attention has been devoted to the study of the excretion of such acids during the period preceding the onset of coma. I desire here to call attention to the appearance of organic acids in the urine previous to the actual development of coma with a view to emphasizing their prognostic significance.

METHODS OF DETECTING THE PRESENCE OF ACIDS IN THE URINE.

In the cases to be reported here, the chief method employed to determine the amount of organic acids excreted has been the process

¹ Read before the Association of American Physicians, May 1, 1901.

² Ueber den Einfluss der Alkalien auf den menschlichen Stoffwechsel. Stuttgart, 1890, also *Arch. f. exp. Path. u. Pharm.*, 1883, xvii, p. 419.

³ *Volkmann's Sammlung klin. Vorträge*, 1889, No. 349-350.

⁴ *Arch. f. exp. Path. u. Pharm.*, 1884, xviii, p. 35.

⁵ *Ibid.*, 1899, xlii, p. 149.

consisting in the estimation of the acids and bases of the urine. In health the urine contains four chief acids united to five different bases, and these acids and bases very nearly neutralize one another. In diabetes, on the contrary, the total known acids of the urine may fail to neutralize the total quantity of known bases and there is an *apparent* excess of bases. The excess of bases over acids is only apparent, for the urine does not contain free alkali. The apparent excess of bases is in reality united to acids, but these acids are not the *known* acids referred to above; they are organic acids and the apparent excess of bases is proportional to the quantity of organic acid which is being excreted. On the basis of these facts, which were first recognized by Stadelmann, Dr. A. J. Wakeman and I have developed a method⁶ of estimating the organic acids of the urine, and this method has been used in the study of our cases of diabetes. It is, of course, to be understood that by this method we do not obtain any clue to the nature of the organic acids, but only a knowledge of their combining power. This knowledge enables us to express the amount of acid present in terms of oxybutyric or any other organic acid, and in references to the quantity of acid excreted in our cases, oxybutyric acid is chosen because it is the preponderating pathological organic acid in cases of diabetes.

The quantity of NH_3 excreted serves roughly to indicate the extent of the excretion of the organic acids. This of course depends on the fact that ammonium is the base chiefly concerned with the removal of organic acids from the body. It is estimated that a daily output of 2 grammes NH_3 corresponds to about 6 grammes of oxybutyric acid, 5 grammes NH_3 to about 20 grammes, and 8 grammes NH_3 to about 36-40 grammes of oxybutyric acid, but I shall show later that considerable amounts of organic acid may exceptionally be excreted without the NH_3 output being above the normal. Nevertheless the method is a valuable one for clinical purposes. We have determined the NH_3 output as part of the process of balancing the acids and bases, but have seldom relied entirely upon it as our evidence of the excretion of organic acids.

In many instances the presence of oxybutyric acid has been ascer-

⁶ *The New York University Bulletin of the Medical Sciences*, 1901, i, p. 7.

tained by obtaining crotonic acid from the urine in the well-known manner.⁷ Comparatively little attention has been paid to diacetic acid on account of its instability.

THE EXCRETION OF ORGANIC ACIDS SHORTLY BEFORE OR DURING COMA.

The urines from several diabetic patients in a state of coma have come under our observation.

Case I. In the first patient, a young girl in the care of Dr. E. G. Janeway, the bases were so much in excess of the acids, in a separate sample of 340 cc. of urine, that the urine was estimated to contain 27.17 gm. of oxybutyric acid in 24 hours.

Case II. In the second case, a woman brought comatose into the City Hospital, without a history, the excess of bases indicated the excretion of more than 10.8 gm. of this acid in the last 24 hours. In the first case the N of NH_3 was 20.88 per cent of the total N; in the second it was 16.73 per cent of the total N.⁸ The second patient came to consciousness after an infusion of sodium bicarbonate, but soon lapsed again into coma. Autopsy showed no lesions which would explain coma except an atrophic pancreas, and the case was regarded as one of diabetic acid intoxication.

Case III. A third case of diabetic coma from which the urine was obtained was in the surgical service of Dr. Markoe in Bellevue Hospital. The small specimen of urine drawn from the bladder after the onset of coma contained 13.5 per cent of N of NH_3 , and the excess of bases indicated an amount of organic acid equal to 9 gm. of oxybutyric acid calculated for 24 hours, assuming an excretion of 10 gm. of N in this period. This is doubtless an underestimate of the quantity of acid.

Besides these three cases of diabetic coma, I have had under observation four cases in which coma appeared several weeks after the recognition of large quantities of organic acids in the urine.

⁷ 200 cc. of urine from which the sugar has been removed by fermentation with yeast are rendered alkaline with sodium carbonate, and evaporated to the consistence of a syrup. To this syrup, in a flask, 15 or 20 cc. of concentrated H_2SO_4 are added; the mixture is now subjected to distillation, the distillation being continued as long as possible. The benzoic acid in the distillate is partially removed by filtering cold. The filtrate is now shaken with about 50 cc. of sulphuric ether. On evaporation of the ether crotonic acid separates out in characteristic crystals which melt between 69° – 71° C.

⁸ The following percentages of N of NH_3 were obtained from the urines of different normal persons living on mixed diet; 5.39, 2.87, 3.94, 4.17, 5.22, 3.25, 4.78, 3.66, 3.45, 2.87.

Case IV. One of these was a patient of Dr. J. S. Ely, of New Haven. On Feb. 26, 1901, the urine for the 24 hours contained organic acids equal to 25.96 gm. of oxybutyric acid, and 20 per cent of the N was present as N of NH_3 . On March 20 (nearly 4 weeks later), the urine contained acids equal to 23.72 gm. of oxybutyric acid, and 21.28 per cent of the N was present as N of NH_3 . In the course of a few weeks this patient died in diabetic coma.

Case V. The second observation relates to a man of 30 (a patient of Dr. H. P. Loomis), who is known to have had glycosuria for at least 6 months, and who had lost considerable weight when the urine was studied, Nov. 1, 1900. On this occasion the excess of bases was equivalent to 24.62 gm. of oxybutyric acid, and 18.45 per cent of the N existed as N of NH_3 . Two weeks later this patient died in diabetic coma after a short period of drowsiness, from which he was temporarily roused by an intravenous alkaline infusion. All the urines from this patient that were examined yielded large amounts of crotonic acid.

Case VI. Another case to be included in this category is that of a child, aged 4, in whom glycosuria was detected about one month before the time of our first analysis of the urine. This glycosuria followed the excessive use of sugars and starches and was at first thought to be of alimentary origin. A 24 hours' specimen was obtained at a time when the child was on a diet moderately restricted as regards carbohydrates. This urine contained an amount of organic acid equal to 7.77 gm. β -oxybutyric acid—a considerable quantity for a child of 4 years. Crotonic acid was obtained but only in small amount. The N of NH_3 reached 19.57 per cent, and the total NH_3 was 1.46 gm. Ten days after the time of this analysis the child suffered from headache and was given 2 grains of calomel. The sugar in the urine fell greatly when the calomel began to act, and a sample was obtained which showed only .031 per cent of sugar as indicated by titration with Fehling's solution. The urine at the time of the previous examination had contained 4 per cent of glucose (42 gm. in 24 hours). An ammonia determination was made to see if there was a corresponding diminution in the excretion of organic acid. It was found that the NH_3 amounted to 2.51 gm. when calculated for the 24 hours, and the N of NH_3 amounted to 28.11 per cent. These figures correspond to a large quantity of organic acid and the observation is a striking example of the independence of the excretion of sugar and organic acids. This patient died in coma one month after the last observation mentioned. Diacetic acid was always present in the last month, and the acetone output was increased.

Case VII. The last observation in this group relates to a distinguished scientist, 52 years of age, under the care of Dr. Osler, who had had diabetes for at least 10 years before he came under my observation. The percentage of glucose had gradually increased from 0.5 per cent to 4 or 5 per cent. It cannot be said that the patient exercised sufficient care in regard to the use of carbohydrates. In June, 1900, after a winter of hard work and worry, he began to have headaches at night and giddiness and diplopia during the day. He also showed debility and excessive irritability. These symptoms followed an indiscretion in diet. The urine for 24 hours ending the morning of June 14 (during the disturbance) contained sugar 5 per cent (70 gm. in 24 hours), less than 0.4 gm. of organic acid (calculated as oxybutyric), 4.61 per cent of N of NH_3 , and only 0.66 gm. of NH_3 . No test was made for acetone or diacetic acid.

On Oct. 9, after a summer of rest, from which there resulted a gain in weight and strength, the urine of this patient contained glucose 4.46 per cent (81.17 gm. in 24 hours), N of NH_3 3.62 per cent, and NH_3 0.629 gm. The organic acids of the urine amounted to 1.8 gm. in terms of oxybutyric acid. The increase in these acids was so small as to be considered of little prognostic significance. Nevertheless, the patient was urged to see Dr. Osler in the hope that he might be induced to take better care of himself. During the end of January, Dr. Osler wrote that our diabetic patient had what appeared to be an influenzal infection, with herpes in the distribution of the 1st division of the 5th nerve. After losing much weight and strength, there was a considerable recovery of weight and some recovery in strength, but after a short interval a boil developed on the leg, the patient became restless, vomited repeatedly and soon died in deep coma. Dr. Fletcher (to whom I am indebted for notes on the progress of the disease) states that there was a definite acetone odor to the breath and that the respirations became of a suggestive Kussmaul type. The following notes relative to the urine were given me by Dr. Fletcher.

Jan. 22, '01. Sp. gr. 1033, sugar 4.2 per cent (polariscope). Amount of urine not stated. Legal's acetone reaction very marked. Typical Gerhard's reaction for diacetic acid. β -Oxybutyric acid 0.2.

Jan. 25, '01. Sp. gr. 1037, sugar 4.6 per cent. Acetone and diacetic acid reaction marked. β -Oxybutyric acid not tested for.

Jan. 28, '01. Sugar 7.9 per cent. Acetone and diacetic acid reactions just appreciable and that is all.

Feb. 17, '01. Sugar 6.25 per cent. No diacetic acid or acetone reactions present.

The seven cases included in this group of diabetic patients agree in that they all show the presence in the urine of large amounts of organic acids. This was demonstrated in most instances by the method of balancing the acids and bases of the urine and by the less reliable but still satisfactory method of simply determining the NH_3 of the urine. In all the cases where an attempt was made to obtain crotonic acid it was found, and we can regard this as satisfactory evidence of the presence of oxybutyric acid in the urine. These results are in accordance with those obtained by Stadelmann, Naunyn, Minkowski and Magnus-Levy from patients either actually in coma or a few days or weeks before the onset of coma. The excretion of acids is often greater before the period of actual coma, for the urine may be considerably decreased in volume at the time of unconsciousness. This was the case in the fourth observation, which has been elsewhere reported in detail by Dr. Ely.

The relatively short time that may elapse between an apparently stationary condition with little excretion of acid and a state of intoxication ending in coma is well seen in Case VII. Analyses made in June and October, 1900, showed the patient to be passing very little organic acid and to be gaining in weight and strength. Diacetic and oxybutyric acids appeared during an acute infection in January, 1901. During a short time the patient gained weight rapidly and the reaction for diacetic acid could no longer be obtained. Then after a new infection diacetic acid reappeared and coma soon developed. Thus the patient died in diabetic coma only six months after the urine was carrying away very little organic acid.

I know of no observation on the urine of a patient in diabetic coma that has failed to show the presence of large amounts of organic acids. Our own results, taken in conjunction with those of Stadelmann, Naunyn, Magnus-Levy and others, show that in diabetic coma and the antecedent period the N of NH_3 is increased to between 16 and 30 per cent of the total N. In one of our cases a small portion of urine, representing only a part of the day's excretion, contained only 13.5 per cent of N of NH_3 , but it is likely that the average for a day would have been higher. In most instances of coma the N of

NH_3 falls between 18 and 25 per cent of the total N. The actual quantity of acid excreted varies enormously. If the kidneys are inactive the quantity may not exceed 10 or 12 gm. of oxybutyric acid. On the other hand, Magnus-Levy mentions an instance in which the amount was 157 gm.⁹

The increase in the excretion of NH_3 is satisfactorily explained on the hypothesis that this NH_3 is diverted from its usual conversion into urea for the purpose of neutralizing the acid which is constantly leaving the cells to pass into the blood.¹⁰

CASES OF DIABETES IN WHICH CONSIDERABLE QUANTITIES OF ORGANIC ACIDS WERE BEING EXCRETED AND WHICH HAVE NOT YET DEVELOPED COMA OR IN WHICH THE OUTCOME IS UNKNOWN.

Case VIII. Perhaps the most instructive case in this group is that of a small and emaciated female of 55, in whom glycosuria had existed for about 2 years at the time of her admission to the City Hospital. The urine on Oct. 13, 1900, contained organic acids equal to 13.15 gm. of oxybutyric acid, and 16.34 per cent of the total N existed as N of NH_3 . The patient was at this time on a free diet containing a large amount of carbohydrates. The diet was restricted in carbohydrates, and on Nov. 1 the urine contained 8.85 gm. oxybutyric acid, with 11.55 per cent of N as N of NH_3 . Crotonic acid was readily obtained. Three weeks later, the diet remaining restricted as regards carbohydrates, the urine contained only 0.98 gm. of organic acid, while the N of NH_3 had fallen to 3.28 per cent. A specimen obtained so recently as March 27, 1901, shows less than 5 gm. of oxybutyric acid in 24 hours, and only 5 per cent of N of NH_3 . The recent urines have yielded only small amounts of crotonic acid.

The instructive features of these observations are, first, that the patient has lived nearly seven months in spite of the fact that the urine contained so much organic acid as to call for 16.34 per cent of N of NH_3 to aid in its neutralization, a percentage seldom observed except in patients on the verge of coma; and, second, the marked falling off in the organic acids which followed a moderate restriction in diet and which has continued in spite of a more liberal diet latterly. The

⁹ *Arch. f. exp. Path. u. Pharm.*, 1899, xlii, pp. 182-3.

¹⁰ This subject is discussed in my paper in the *Trans. Assoc. American Physicians*, 1900, xv, p. 236.

patient has become very much emaciated and is often somnolent during the day.

Case IX. A similar observation was made on a corpulent man, aged 45, who has had glycosuria for ten years. For many years the rigid exclusion of carbohydrates from the diet was promptly followed by the disappearance of sugar from the urine, but for more than a year the glycosuria has persisted in spite of the severest restriction in diet. On March 18, this patient passed 3700 cc. of urine containing 4.7 per cent of sugar, and the equivalent of 9.26 gm. of oxybutyric acid. After 4 days on a restricted diet, 2380 cc. of urine were passed with 2.75 per cent of sugar and only one gm. of oxybutyric acid. Although the patient continued to live on a somewhat restricted diet, the acids increased to 10.7 gm. of oxybutyric acid in less than one week from the time of the previous observation. The first of the three urines mentioned yielded crotonic acid, and there is no doubt that at least part of the organic acid excreted consisted of oxybutyric acid.

It is of interest that no diacetic acid could be detected in the urine just mentioned, although the examination was promptly made.

An exceptional and highly noteworthy feature of this case is the behavior of the nitrogen of ammonia. The first analysis gave 2.42 per cent of N of NH_3 , with a total of 0.82 gm. of NH_3 . The second analysis gave 3.27 per cent of N of NH_3 , with a total of 1.08 gm. of NH_3 . The third analysis gave 4.14 per cent of N of NH_3 , with a total of 1.31 gm. of NH_3 . These values all fall within the limits of the normal excretion of NH_3 . This is noteworthy because the patient was excreting a considerable amount of organic acid, and it is a well-established fact that NH_3 is the principal base to which the organic acids are united when they are excreted. It is clear that in this instance the organic acid must have been united to some other base than NH_3 , and there is reason to think that the base is chiefly potassium.¹¹

The excretion of the NH_3 , the Na and the K, during and before and after the period of restricted diet, is indicated in the following table.

¹¹ This observation is not entirely conclusive as to whether the base removed by the organic acid was chiefly K or Na, for in our method of balancing the acids and bases of the urine these bases are not determined directly. In this method we assume that the Na of the urine corresponds closely to the Ca excreted and the K is obtained by difference, on the basis of this assumption. The results must therefore be regarded as only approximating the truth.

TABLE I.
Excretion of NH_3 , Na, and K in Case IX. (24 hour periods.)

Date.	Diet.	Excretion of organic acid in terms of β -oxybutyric acid.	Excretion of NH_3 .		Excretion of Na_2O in terms of Na.	Excretion of K_2O in terms of Na.	Excretion of Sugar.	
			Total.	Per cent. N of NH_3			Total.	Per cent.
Feb. 18, 1901.	Carbohydrates moderately restricted.	9.26 gm.	0.82 gm.	2.42	6.69 gm.	5.10 gm.	174. gm.	4.7
Feb. 26, 1901.	Carbohydrates excluded during 4 days.	1.0	1.08	3.27	7.08	3.32	65.45	2.75
Apr. 1, 1901.	Carbohydrates moderately restricted.	10.7	1.31	4.14	4.98	4.82	101.2	3.68

The urine on which the first observation was made shows the excretion of K_2O , in terms of Na, to be nearly as great as the excretion of Na_2O in terms of Na. These are not normal conditions, since when a normal individual is eating plentifully, as in this instance, the Na_2O is only about one-half as abundant as the K_2O . In the second observation the K_2O has fallen to less than one-half the Na_2O , with very little change in the total Na_2O . This fall in K_2O corresponds to the period of strict diet and small excretion of organic acids, that is to the period in which there is no unusual demand for bases to neutralize acid. In the third observation the organic acids have again increased to considerable proportions and the K_2O equals the Na_2O . We are led to the conclusion that it is chiefly the K which is concerned with the removal of the organic acid.¹² If we had depended in this case on the study of the excretion of NH_3 to determine whether there was any pathological excretion of organic acids we should have reached the conclusion that such acids were not being excreted. Probably the true condition could only have been shown by balancing the acids and bases as was here done, since diacetic acid was not detected and the yield of crotonic acid was very small, and in one instance even questionable.

The patient to whom reference has just been made is slowly losing weight, but is still a well-nourished and vigorous individual, capable of doing exacting physical labor. The fact that the exclusion of carbohydrates no longer suffices to stop the glycosuria indicates a loss of assimilative capacity as compared with the conditions one year ago.

¹² See preceding footnote.

The clinical indications are that this patient is likely to do well for many months in spite of a considerable excretion of organic acids at times.

Case X. The next case in this category is that of a middle-aged man, who came under the observation of Dr. E. K. Dunham. He first noticed the excessive amount of his urine about April 7, 1900. Between April 1 and May 1, 1900, that is in one month, he had lost 50 pounds, but still weighed 147 pounds. He suffered from great thirst, anorexia, and great debility, but was able to bring a large jug of his urine from Englewood, N. J., to the Carnegie Laboratory, carrying it part of the way. The urine for 21 hours amounted to 11,500 cc., and contained organic acids equal to 30.02 gm. of β -oxybutyric acid in 24 hours. The N of NH_3 equalled 17.55 per cent, and the total N of NH_3 was 3.32 gm. The ferric chloride reaction for diacetic acid was strong. In the 24 hours' urine the sugar reached the enormous amount of 556.3 gm. The amount of oxybutyric acid excreted makes it probable that this man was not far from a fatal termination in coma, and it is most unfortunate that the subsequent history of the patient is unknown.

Case XI. A somewhat similar but less acute case came under the observation of Dr. G. A. Spalding at St. Luke's Hospital. A man, aged 28, lost 93 pounds in three months, and for six weeks previous to the analysis passed large amounts of urine. At the time of the examination the chief complaints were debility and great thirst. The urine for a portion of the 24 hours contained 6.01 gm. of oxybutyric acid. The quantity for 24 hours was probably two or three times this amount, judging from the N excretion. The N of NH_3 reached 9.7 per cent of the total N. Crotonic acid was easily obtained from the urine. The patient continued to lose weight and strength, but the ultimate outcome is not known.

Case XII. The last observation to be included in this category relates to a male patient of Dr. C. C. Ransom, 75 years of age, who has had persistent glycosuria for several years. The general health is good and the patient is said to be without symptoms. On Nov. 4 the urine contained an amount of acid equal to 6.9 gm. of oxybutyric acid, with 7.08 per cent of N of NH_3 and 0.98 gm. of NH_3 . The urine contained 1.17 per cent of sugar, there being 22.81 gm. in 24 hours. The patient has been in excellent health since this observation was made, and has been on a diet moderately restricted in carbohydrates. On Feb. 19, 1901, the urine contained 0.32 per cent sugar and only 2.2 gm. of oxybutyric acid with 3.31 per cent of N of NH_3 .

Perhaps the most important fact brought out by the cases in this group is that a diabetic (Case VIII) may live many months after the appearance of a very high percentage of N of NH_3 in the urine. Sandmeyer's reports on the cases studied and published with Külz show some remarkable illustrations of this fact.¹³ One patient (Case 414) died 11 months after the urine contained 3.74 gm. of NH_3 (average of 3 different urines); another (Case 415) lived 15 months after the urine showed 7.23 gm. of NH_3 —an enormous quantity. Other treatment than restriction in diet seems to have had little to do with these remarkable instances of prolongation of life. In my Case VIII the patient showed no improvement in symptoms when the alkali treatment was instituted.

CASES OF DIABETES IN WHICH THERE IS EITHER NO PATHOLOGICAL EXCRETION OF ORGANIC ACIDS OR IN WHICH THE EXCRETION IS SMALL.

The cases (Table II) which fall into this group are, with two exceptions (Cases XII and XIV), examples of diabetes of mild form; that is, the patients have none of the obtrusive clinical evidences of diabetes such as thirst, polyuria, and loss of weight and strength. In Case XIV an alcoholic neuritis complicated the condition, and the sudden death was probably connected with the extensive myocardial changes. In cases XVI, XVII, XVIII, XIX and XX the prospects for life appear to be good. Case XIII is one in which the patient is constantly losing weight and is doing badly in other respects. The excretion of organic acids is so small that the unfavorable symptoms cannot be brought into relation with them. Case XV has also progressed unfavorably, with an inclination to somnolence, in spite of the small excretion of acids. In Case XVIII the patient excreted 8.36 gm. of acid in terms of β -oxybutyric acid, but crotonic acid was not positively detected. We cannot, however, feel certain that the urine contained no β -oxybutyric acid because of the imperfections in the methods of obtaining crotonic acid from urine. This case might perhaps have been included in the second group.

¹³ E. Külz. *Klinische Erfahrungen über Diabetes*, Jena, 1899.

TABLE II.

Date.	Clinical notes.	Acid in terms of β -oxybutyric acid.	NH ₃		Glucose.		No. of case.
			N of NH ₃	Total NH ₃	Total.	Per cent.	
1900. May 8	Female, 35 yrs., glycosuria 6 years, loss of weight, thirst, nervousness, cardiac weakness.	1.85 gm.	4.98%	gm. 1.294	gm. 199.6	2.9	XIII.
Dec. 5	Same patient, has continued to lose weight and strength.	None	4.24%	0.539	125	4.1
Oct. 5	Woman, 50 yrs.; alcoholic neuritis, duration glycosuria unknown. Alcoholic delirium. Died suddenly Nov., 1900. No coma. Interacinar pancreatitis.	1.85 gm.	6.29%	0.58	34.23	3.4	XIV.
Nov. 12	Woman, 75 yrs.; glycosuria 6 years, 3-4% sugar on repeated examination. Moderate loss of weight, debility, slight somnolence.	1.62 gm.	4.08%	(Average 3.4)	XV.
Nov. 22	Man, 30 yrs.; strong, well nourished, glycosuria for 2 years. Was "cured" at Bellevue Hospital 2 years ago. Drinking hard for 2-3 weeks.	None	5.52%	0.12	208	4.16	XVI.
1901. Feb. 23	Male, 54 yrs.; glycosuria 10 yrs.; from 1-4% glucose. Formerly controlled by exclusion of carbohydrates. Loss of 10 pounds in three weeks owing to restricted diet. Now gaining.	7.05 gm.	3.70%	1.43	0.39	XVII.
Mar. 7	Same patient. Carbohydrates excluded from diet for 5 days previous.	1.03 gm.	3.0%	1.0	0.06
Mar. 28	Same patient on moderately restricted diet, gaining in weight and strength.	1.13 gm.	4.14%	1.3	0.88
Feb. 24	Male, 61 yrs.; glycosuria 4 yrs.; 1-2%. Disappears when carbohydrates are stopped. General health excellent. Inclination to corpulency, no loss in weight. Carbohydrates restricted.	1.4 gm.	4.42%	0.81	46.48	2.8	XVIII
Apr. 6	Same patient on a diet only moderately restricted in carbohydrates. General condition is excellent.	8.36 gm. No crotonic acid	4.98%	0.91	22.62	1.56
Apr. 25	Same patient. Diet almost free from carbohydrates.	0.34 gm.	3.85%	0.67	3.8	0.25
May 7	Same patient. 5th day on diet entirely free from carbohydrates, —meat, fat, fish, string beans.	0.84 gm.	4.21%	0.70	0.2 or less
May 12	Same patient. 10th day on very strict diet.	No pathological acids, i. e., acids in apparent excess of bases	5.08%	0.78	Less than 0.1	Less than 0.1
Apr. 6	Female, 60 yrs.; glycosuria for several years, diet only slightly restricted as regards carbohydrates. No symptoms, well nourished.	3.2 gm.	4.75%	0.69	0.1	XIX.
May 7	Same patient. Diet unrestricted	2.2 gm.	3.89%	0.552	4.5	0.3
May 11	Same patient	No pathological acids, i. e., acids in apparent excess of bases	4.82%	0.585	3.3	0.33
May 21	Female, 63 yrs.; general health fair, spare build, rather thin, chronic gout. Glycosuria followed influenza in January, 1901. In April sugar 3.85%, 3.69%, 2.99%, May 19, 1.1%.	No evidence of pathological acids	3.01%	Less than 0.13	XX.

THE RELATION BETWEEN THE EXCRETION OF ORGANIC ACIDS AND THE
EXCRETION OF GLUCOSE.

An examination of the figures relating to the excretion of acids and of sugar makes it quickly evident that the fluctuations in these constituents of the urine are usually by no means proportional. Thus in Case VIII we find the following conditions:

	Sugar.		Acids.
	Per cent.	Total.	
Oct. 13, 1900	3.57	110.7 gm.	13.15 gm.
Nov. 20, 1900	4.54	147.6 "	0.98

Here we see a marked increase in the sugar excreted with a very striking fall in the quantity of acid.

In Case IX we find somewhat different conditions, as shown in Table I on page 625. In this instance there was a considerable fall in the sugar, but the percentage of decrease in the acids was considerably greater.

Again in Case XVII, although there was a fall, nearly proportional, in sugar and in acids, from Feb. 23 to March 7, this was followed on March 28 by a rise in the excretion of sugar, while the acids remained little changed. It is worthy of note that in each of these three instances the considerable fall in the excretion of acids followed a restriction in carbohydrates of the food. This fact appears to me to be of considerable practical interest. If a patient who is eating carbohydrates liberally has a considerable quantity of organic acids in the urine, there is the chance and even the probability that a restriction in diet will be followed by a considerable fall in this excretion of acid. If, however, a patient passes the same quantity of acid as in the case just mentioned, in spite of the fact that he is getting no carbohydrates, the outlook is likely to be worse than in the former case. In other words, we must test the effect of diet upon the acids just as we test its effect upon the excretion of sugar. Other conditions remaining the same, a quick fall in the acids, in response to diet, is to be considered a favorable indication, while the persistence of a considerable excretion of acid, in spite of diet, is to be considered unfavorable.

It sometimes happens that considerable sugar is excreted when there is little excretion of acid. Thus in Case VII we find on one

occasion an excretion of 70 gm. of sugar with less than 0.4 gm. of acid, and on another occasion 81.17 gm. of sugar with 1.8 gm. of acid. On the other hand, in Case XIX we find only 22.62 gm. of sugar with 8.36 gm. of acid.

Notwithstanding these discrepancies, it is true that large amounts of organic acids generally accompany large amounts of sugar, and conversely that a large excretion of sugar (more than 200 gm. per day) is usually associated with a considerable excretion of organic acids. A small excretion of sugar (25 gm.) is rarely accompanied with considerable amounts of organic acids, but on the other hand, small quantities of acid often go with considerable amounts of sugar (75-200 gm. per day). (See Cases XIII and XVI).

A striking example of the independence of the sugar excretion and the acid excretion has already been referred to in connection with Case VI. Here the excretion of acids, as indicated by the N of NH_3 , was very large at a time when the sugar almost disappeared from the urine.

THE NATURE OF THE ORGANIC ACIDS IN THE URINE AND BLOOD OF DIABETES.

Since the independent and nearly simultaneous discovery by Minkowski¹⁴ and by Külz¹⁵ of β -oxybutyric acid in the urine of diabetics, many observations have been made which leave no doubt that when the NH_3 content of the undecomposed urine of diabetes is markedly increased, the presence of β -oxybutyric acid can always be demonstrated. There are two properties of this acid which can be utilized for its detection. One is the formation of crotonic acid under the action of strong sulphuric acid and heat. The other is the levorotary action of the acid.¹⁶ By the use of methods based on these properties, it has been possible to show that most of the organic acid in the urine of patients in coma or in the pre-comatose state is β -oxybutyric acid. Although diacetic acid is probably never absent under these conditions, there is reason to think that it is never present in the large amounts in which oxybutyric acid occurs. Of other acids in

¹⁴ E. Minkowski, loc. cit.

¹⁵ *Zeitschr. f. Biologie*, 1884, xx, p. 165.

¹⁶ The acid can also be extracted directly from the urine.

the urine in diabetes we know little. It seems by no means improbable that other organic acids are sometimes associated with β -oxybutyric and diacetic acids.

As yet there have been few observations on the blood and tissues of diabetics which throw light on the nature of the acid in the blood. It is natural to suppose that as the urine contains so much β -oxybutyric acid in diabetes, the blood must also contain it. The acid has indeed been discovered in the blood by such careful observers as Minkowski and Magnus-Levy. The quantity reported by Minkowski is more than 2.2 parts per mille. Magnus-Levy found the acid in the liver, spleen, muscles, brain and gastric contents. It is of interest that considerable acid was found in the brain. I was unable to obtain crotonic acid either from the liver, blood, or brain of a patient who died in diabetic coma, but do not consider that the presence of β -oxybutyric acid can be excluded in this case.

The discussion of the origin of the organic acids formed in diabetes and their relation to diabetic coma I shall reserve for another publication. I desire to express my indebtedness to Dr. A. J. Wakeman for making most of the analyses on which this paper is founded.

CONCLUSIONS.

It seems desirable to emphasize the following conclusions:

1. A careful balancing of the normal acids and bases of the urine makes it possible not merely to detect the presence of organic acids in the urine, but also to determine approximately the amount of such acids. The method recently described by Herter and Wakeman can be recommended as securing a greater degree of accuracy, for the amount of labor involved, than any other procedure.

2. The determination of the N of NH_3 is a useful procedure for clinical purposes, since it is probably true that a considerable excretion of organic acid (say 15 gm. oxybutyric or more in 24 hours) is always attended by an increased excretion of NH_3 . As much organic acid as corresponds to 10 gm. oxybutyric acid may be excreted in 24 hours without causing an increased excretion of NH_3 (Case IX). We cannot therefore rely on the ammonia output to detect moderate quantities of organic acid.

3. Where organic acids are removed in considerable amount without increasing the excretion of NH_3 , the acid takes out other alkalies, probably in some instances chiefly K.

4. In cases of diabetic coma the urine always contains a large excess of organic acids and the N of NH_3 is usually increased to 18 to 25 per cent of the total N.

5. Crotonic acid can regularly be obtained from the urines of patients in diabetic coma.

6. The condition of diabetic coma is preceded by a period of days, weeks or months, in which there is a large excretion of β -oxybutyric acid (20 gm. or more in 24 hours), and in which the N of NH_3 is largely increased.

7. Patients whose urines show or have shown a large excretion of organic acids are in danger of developing diabetic coma, but the N of NH_3 may temporarily rise as high as 16 per cent and yet coma may be delayed for more than 7 months (Case VII). The persistent excretion of more than 25 gm. of β oxybutyric acid indicates impending coma.

8. A patient passing 30 gm. of β -oxybutyric acid in 24 hours may still have enough energy and strength to be about all day and perform considerable muscular work (Case X).

9. A patient who has been excreting very little organic acid and has gained weight may within a few months show the presence of considerable quantities of organic acid, and die in typical diabetic coma (Case VII).

10. When the urine contains little or no organic acid there is no immediate prospect of diabetic coma, but patients with such urine are probably liable to most of the other dangers that threaten diabetic patients. The relation between the degree of acid intoxication and the susceptibility to infection seems worthy of special experimental study.

11. Where the urine regularly contains more than 200 gm. of sugar per day there is usually considerable organic acid in the urine and large amounts of acid, indicative of coma, are invariably accompanied by considerable or great glycosuria.

12. Sometimes there is much sugar and little or no acid in the urine, and sometimes there is considerable acid and little sugar. These facts render it desirable to examine the urine of diabetic patients at least once a month with reference to the amount of acid excreted, for the element of acid intoxication must be clearly separated from the element of glycosuria in our study of the progress of a case. In other words, we must recognize the acid intoxication as an important and sometimes as a dominant factor in the prognosis, and this element should be regarded even in those cases of diabetes which have the clinical indications of a mild type of the disease. We may thus hope to prolong life in many instances by taking precautions, as to diet and out-of-door life, which might not otherwise be deemed necessary.

13. The withdrawal of carbohydrate food frequently leads to a considerable reduction in the quantity of organic acids excreted. The reason for this is not yet clear and the phenomenon deserves careful study.

NOTES UPON THE AGGLUTINATIONS OBTAINED BY
INTRAPERITONEAL INSERTION OF CELLOIDIN CAP-
SULES CONTAINING BACILLI AND UPON A MODE
OF PREPARING SUCH CAPSULES.

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While carrying out some studies upon agglutinations by the usual method of injecting bacilli, living or dead, into the animal tissues, I was led to try a method of enclosing the bacilli in capsules, which were inserted into the abdominal cavity of the animal, and I found that by this method also the serum gained agglutinative power. The extent and the other characters of such agglutinations will be dealt with in later paragraphs, but before recording my results, it is well, I think, that a few words should be said with regard to the preparation of the capsules. For although Nocard and Roux's¹ work on the micro-organism of pleuro-pneumonia of cattle, and Nocard's² experiments on the transformation of human into avian tubercle bacilli, by placing them in sealed capsules within the fowl's peritoneal cavity, have brought increased attention to this method of growing bacteria within the body, free from the intervention of the body cells, there does not exist, to my knowledge, in bacteriological literature, any detailed account of a satisfactory method of preparation of the same, and if I mistake not, the difficulty in making capsules which will not rupture nor leak, has been found so considerable that the method has by many been taken up only to be abandoned.

The idea of using collodion or celloidin capsules is, bacteriologically speaking, of comparative antiquity; quite early in the nineties, a somewhat primitive capsule was employed in the Pasteur Institute by

¹ *Ann. de l'Institut Pasteur*, 1898, xii, p. 270.

² *Ibid.*, 1898, xii, p. 561.

Metchnikoff and others. This was formed by taking a glass rod or pencil, dipping it into collodion until the desired thickness of coating was obtained; the capsule was then stripped off the rod and the culture inserted, the mouth tied, and finally, the interstices at the neck were cemented over with collodion. The procedure was first mentioned in an article by Metchnikoff, Roux and Salimbeni.³

The defects of this method are obvious: namely, the amount of manipulation required, the long time the capsule has to be kept exposed to contamination while the mouth is being sealed up and the liability for the new coat of collodion at the neck not to adhere thoroughly to the previously dried material. Gradually, it would seem, the method of making these capsules was improved in Paris, though I can find no clear statement of how the capsules were made by Metchnikoff, Roux, and Nocard in their later work.

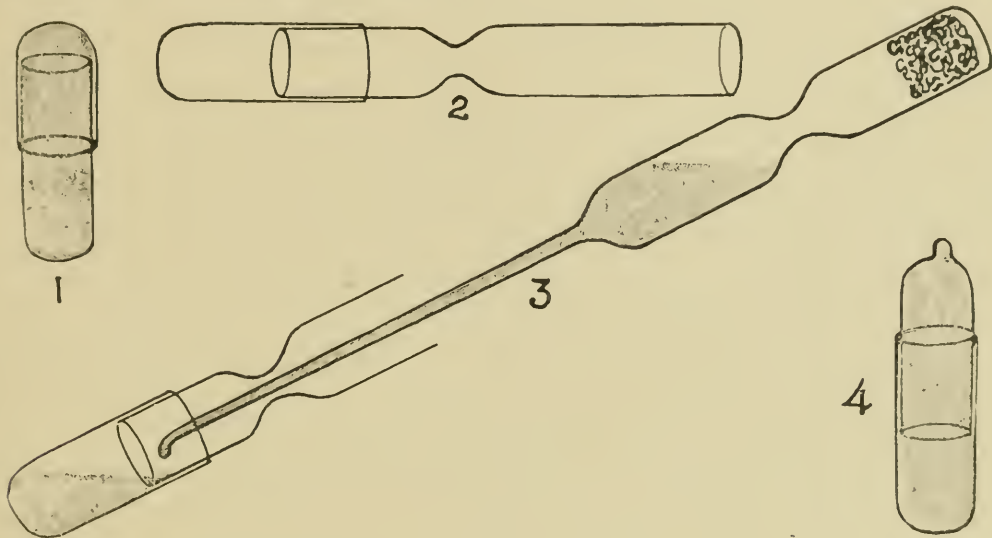
A distinct step in advance was made upon this side of the Atlantic some two years back, so far as I have been able to trace, by Dr. Prudden and others in the Laboratory of the College of Physicians and Surgeons of New York. It consists in employing the gelatin capsules now obtainable at any druggist's, and used for the administration of unpleasant drugs. These capsules are taken as a framework and coated with celloidin; next, the gelatin is dissolved out in a sterile test tube, the cap being then luted upon the body of the capsule by means of painting with thin celloidin. We obtained a knowledge of this method from Drs. Trudeau and Baldwin at the Saranac Lake Laboratory, and, if we are not mistaken, yet further advance was there made by replacing the gelatin cap with a length of glass tubing to which the body of the capsule was luted, the tube being sealed in the flame after filling the capsule. At Saranac Lake they employ a bulb-shaped capsule, and from what I learn from Dr. Baldwin, some little difficulty has been experienced owing to the tendency of capsules so made to undergo rupture within the body and thus set up a general infection.

The method described below has proved so simple and at the same time so successful that a detailed account of it may furnish means to

³ Toxine et antitoxine cholérique. *Ann. de l'Institut Pasteur*, 1896, x, p. 257.

others of adopting this manner of passing bacteria through the animal body without coming into direct contact with the tissues. The early methods have been touched upon lest I be thought to claim in the slightest degree the credit due to originality.

Celloidin is especially adapted to this work, as it prevents the escape of the organisms while it allows osmosis of the fluids to go on freely. Enclosed in the celloidin capsule the bacilli lie exposed to the body fluids, their soluble products have free egress to the tissues, and, should observations upon the bacilli themselves be desired, the capsule can be removed with a certain knowledge that it contains the form that was originally introduced.



1. Gelatine capsule, natural size.
2. Diagram of glass tube with adherent capsule.
3. Method of filling the capsule.
4. Capsule filled and sealed ready for introduction into the peritoneal cavity; natural size.

A piece of glass tubing, 1 cm. in external diameter and 6 to 8 cm. long, is taken and a narrow and rather abrupt neck is drawn upon it about 3 cm. from one end. It is well, as a matter of precaution, to round off the edges of this end in the flame, lest any sharp edge should later, on manipulation or movement of the capsule within the body, cut through the celloidin. Then over this end, after heating it slightly, the body of a gelatin capsule is fitted, the top being discarded; the hot glass melts the gelatin and there is immediate adhesion. The accompanying diagram 1 gives the exact shape and

size of this capsule with its top. The advantage of this form over the previously mentioned globular forms with neck is that in the latter there is danger of rupture where the neck joins the body, and this danger is done away with here. The glass tubing should pass without difficulty into the capsule (2 in the figure), thereby providing to some extent against the slight shrinkage which may occur during sterilization. The capsule is now repeatedly dipped in thin celloidin, care being taken to dip well beyond the upper edge of the capsule, so that the celloidin adheres directly to the glass; between each fresh coat the capsule is allowed to dry, the dipping being continued until the layer of celloidin is judged to be sufficiently thick.

It is now necessary to melt out the gelatin. I used to accomplish this by first pouring some water into the capsule and then placing the whole in a sterile test tube, with the open end of the glass tube downwards, and heating it in the autoclave for 20 minutes. Lest the melted gelatin refuse to run down the sides of the capsule, a thin wire or fine broom straw may be inserted through the neck of the tube. By this method I obtained a thoroughly practicable capsule and could keep it under sterile conditions without difficulty until it was needed for use. The capsules did, however, exhibit some tendency to shrink, and as shown to me by Dr. C. H. Higgins (who in this laboratory has employed the capsules in his studies upon the tuberculosis of cattle), this shrinkage may be prevented. The modification is as follows:

The capsules are filled with water and placed in test tubes themselves half filled with water, and these are then heated in the autoclave or steam sterilizer. Capsules so treated retain their form admirably, and after emptying out the melted gelatin and half filling with water they may be sterilized and kept, with the open end upwards, in a sterilized test tube containing either water or broth until they are needed.

Into the capsule thus prepared a culture is inserted by means of a fine Pasteur pipette, which is sufficiently small for the capillary tube of the same to pass through the narrow neck (3 in figure). Care must be taken that the inside of the neck is not wetted by the culture,

for if it should be the glass will probably crack during the sealing of the capsule. This step is accomplished by removing the capsule from the test tube by means of a pair of sterile forceps and fusing the narrow neck of the tube rapidly in the blow-pipe flame (4 in figure). The capsule thus sealed must be replaced in the sterile test tube until needed for use. By this wet method of keeping the capsules there is so little danger of contamination that they may immediately be inserted into the peritoneal cavity or elsewhere. When using the dry method, to make sure that the capsule is intact, it is well to keep the sealed capsule in a broth tube for 24 hours, when, if the broth remains clear, the operation may be performed. Nevertheless, one becomes so proficient, that after the first few attempts I was able to use the capsules immediately, and upon subsequent removal from the body found that they were entirely free from any evidence of bacterial growth externally. Until a worker has perfected his technique, it certainly must be laid down that the filled capsule should be preserved in a broth test tube for 24 hours before being placed within the tissues.

The abdomen of the animal is opened antiseptically, the capsule inserted and the wound closed. I have generally found on removal that the capsule has slipped around freely in the cavity; sometimes, however, it is surrounded by adhesions. If it is desired to remove the capsule without killing the animal, the chief practical difficulty lies in finding and recognizing it through the small wound by touch. When found it can be placed in a sterile tube and opened by sterile forceps or scissors.

The agglutinative results, spoken of above, were observed while passing through rabbits some forms of the paracolon group, obtained from Drs. Harvey Cushing, Gwyn and Harris, of the Johns Hopkins Pathological Laboratory and Hospital, Baltimore; their cultural characteristics were tested and found to correspond to *Bacillus O* (Cushing),⁴ *B. paracolon* (Gwyn),⁵ and *B. enteritidis* (Gärtner). They were inserted in the capsules as 24- or 48-hour bouillon cultures.

The fact elicited in the course of these experiments was that for a

⁴ *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 156.

⁵ *Ibid.*, 1898, ix, p. 54.

few days after the insertion of the capsule, no agglutinative reaction of the serum appeared, even in dilutions of 1 in 10; but on the 10th to the 15th day it began to appear, first in dilutions of 1 in 10 and 1 in 20, gaining in potency daily, until by the 19th to 21st day it would attain a potency of 1 in 1000. Upon the removal of the capsule the agglutinative power decreased day by day, with the same speed with which it had arisen. The agglutinative power of the serum was restricted to the variety of bacillus contained in the capsule, and did not extend to the other, apparently closely related, forms, except in one single instance. If two capsules containing different organisms were put in the same animal, the animal's serum was found to have an agglutinative power over both at the same time, and to about the same degree of potency.

The facts of the experiments are here briefly summarized:

(1) *Rabbit*, with intraperitoneal capsule containing *B. paracolon* (Gwyn).

Serum: Positive to *B. paracolon* (Gwyn), on 8th day, 1 in 10, increasing to 1 in 80 on 15th day. Negative to *Bacillus O*, *B. chol. suis*, *B. icteroides* (Sanarelli), *B. icteroides* (Reed), *B. enteritidis* (Gärtner), *B. morbificans bovis*.

(2) *Rabbit*, with intraperitoneal capsule, 1st *B. paracolon* (Gwyn), 2d (after removal of 1st), *B. icteroides* (Reed).

Serum: Positive to *B. paracolon* (Gwyn) and *B. icteroides* (Reed), and negative to all the others.

(3) *Rabbit*, with capsule of *B. enteritidis*.

Serum: Positive to *B. enteritidis*, 1 in 10, on 11th day, increasing to 1 in 1000 on 21st day; capsule removed on 26th day; agglutinative reaction had fallen to 1 in 500 on 31st day. Serum negative throughout to all the other above-named forms.

(4) *Rabbit*, with capsule of *Bacillus O*.

Serum: Positive to *Bacillus O* on 13th day; increasing. Positive to *B. icteroides* (Sanarelli) in 1 in 10; apparently not increasing. Negative throughout to others.

These results will be seen to bear out Cushing's results in his experiments upon *Bacillus O*, that inter-agglutinations do not necessarily occur between closely related varieties of bacilli.

The above observations do not represent all my studies upon the effects of introducing bacteria in capsules into the peritoneal cavities

of rabbits. They give, however, the results obtained with reference to the agglutinating properties of the blood serum of these animals. I had intended to make the series more complete, but my departure to South Africa arrested my work along these lines at this point. So far as they go, the results obtained by me were so constant and so definite that I feel that I am justified in publishing this note and even in drawing certain conclusions from the results obtained.

Of these results, that which is most obvious is that agglutination, however produced, would appear to be strictly associated with the existence of the bacteria in a living state within the body, for otherwise we cannot explain the fact that removal of the encapsulated bacteria from the peritoneal cavity is followed by the steady disappearance of the agglutinating property of the serum of the animal.

So far as these observations go, they would appear to explain the continued existence of the reaction for months and years in some individuals following upon an attack of typhoid and the rapid disappearance of the reaction in other cases, and this by the continued existence of the bacteria within the tissues in one set of cases and by their complete destruction in the other series. A few years ago this conclusion would have seemed impossible, but now-a-days we are, I think, prepared to accept it, for it is now a familiar experience that typhoid bacteria may be obtained from the gall bladder many months after the patient has apparently wholly recovered from the acute disease, while similarly, long months after the patient has been subjected to the disease we occasionally encounter the bacilli in pure culture in abscesses in the neighborhood of joints and other lesions, observations which prove absolutely that the bacilli may continue for long periods, either lying latent or proliferating very slowly in some one or other region of the body.

In attempting to form a satisfactory theory of agglutination, we possess data which lead us to suppose that the bacteria in culture form certain agglutinins which unite with certain other agglutinins which are the product of the tissues. (The normal serum will, in certain cases, produce in low dilutions an agglutination; but this power is probably not an inherent quality of tissues or serum, but is

caused by the presence of a subinfection by a bacillus nearly related to the bacillus with which the agglutination is made. So rarely does this agglutination by apparently normal serum occur that it may be disregarded.) The fact remains that the tissues, reacted upon by an infection, respond by the production of an agglutinin. By the capsule method we are able to assert that the agglutinin produced by the tissues is not a reaction to the bacillary bodies, nor yet a reaction of the kind called inflammatory, but a reaction to the chemical bacillary products or a combination of the serum with these chemical products.

SUMMARY.

1. Capsules made as described above allow dialysis, when placed in the peritoneal cavity.
2. The normal tissues, unstimulated, do not possess the power of causing agglutination; they do not require to be stimulated by the presence of the bacterial bodies, but will produce their share of the agglutinins when acted upon by the bacillary products.
3. Agglutination follows the insertion, in the peritoneal cavity, of "capsuled" bacilli; it gradually increases in degree, and on the removal of the capsule containing the bacilli, begins to disappear.
4. Varieties of bacilli, related closely in morphology and cultural reactions, do not, as a rule, produce serums which inter-agglutinate.

SOME EXPERIMENTAL DATA ON THE SIGNIFICANCE OF CONCENTRATION AND OF MULTIPLICITY OF AREA IN HYPODERMIC INJECTIONS.¹

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In the theoretical and practical studies of the effects of bacterial toxins and antitoxins upon the animal body, the subcutaneous method of application is extensively employed. Therefore any contribution to the knowledge of the precise working of this method ought to be of interest to bacteriologists. This is my excuse for bringing before this society a subject which in itself is not an integral part of bacteriology.

In a recent communication by v. Czyhlarz and Donath,² the assertion was made that the animal tissue is capable of neutralizing or fixing the poisons of strychnine and venom. The fundamental experiment from which this assertion was derived was as follows: The thigh of a guinea-pig was tightly constricted and a fatal dose of strychnine injected into it at a point peripheral to the ligature. When after an hour or two the ligature was removed, the characteristic effect of strychnine did not set in. In a series of experiments by Langmann and myself,³ it was shown, as I believe conclusively, that this failure is not due to any capacity of the tissue to neutralize poisons, but to the impairment of the capacity of absorption of the constricted leg. One of the experiments which was made in support of our view was as follows: Instead of injecting the minimum effective dose, which is for a guinea-pig of 250 grammes about 1.5 mgr., into one leg, we divided the dose into three parts and injected them in

¹ Read at the meeting of the Society of American Bacteriologists, Dec. 28, 1900, Baltimore.

² *Centralbl. f. innere Med.*, 1900, p. 321.

³ Meltzer and Langmann, *Medical News*, 1900, lxxvii, p. 685.

three extremities which had been constricted prior to the injections. We had here in comparison to the other experiment a greater amount of tissue to a smaller amount of poison, and if the tissues were capable of neutralizing the poison, there should have been surely in this experiment no effect of strychnine visible. Instead, no sooner were the ligatures taken off than violent convulsions broke out. Our explanation was, that there were for the same amount of poison in the three legs more paths of absorption open than in the one constricted leg, hence the positive result.

One observation which I made in the last-mentioned experiment, gave the stimulus to the new series of experiments, the results of which I shall report here briefly. The tetanus, which followed the removal of the ligatures of the three legs, was more violent and set in sooner than in the case in which the sum of the three quantities was injected into one non-ligatured leg. It was now a question, whether the distribution of the same quantity of the solution of strychnine to several areas of the body does not indeed favor a more rapid absorption. The characteristic effect of the poison appears the more quickly and the more pronounced, the more quickly and the more abundantly it is absorbed into the blood and from there into the central nervous system. The absorption from the subcutaneous tissue takes place either through the blood-capillaries or through the lymphatics or in both ways. Now the distribution of the same quantity to several areas brings the poison, it would seem indeed, into contact with a greater number of capillaries and lymphatics, and it would therefore be quite plausible to assume that such a distribution facilitates absorption. On the other hand, the larger the quantity which is injected subcutaneously into one place, the greater is the pressure which it exerts upon the surrounding tissue. And as pressure facilitates filtration, and filtration is at least one of the factors of interstitial absorption, we might suspect that by the distribution one of the factors favoring absorption becomes impaired. Here was a problem and I tried to solve it by experiment. I employed strychnine again, on account of the characteristic reaction and the short time in which a decisive response can be obtained. In the comparison I noted of

course also the length of the interval which elapses between the injection and the appearance of the tetanus and the degree of violence of the latter, but in my positive conclusions I relied chiefly upon the appearance and non-appearance of a tetanus. The following sample experiments illustrate the result:

Rabbit, 1700 grammes, injected into one leg 1 mgr. of strychnine. No effect. Five days later, injected into the same animal 1 mgr. distributed among three extremities. The first tetanus 23 minutes after injection, followed by a few more; animal survived.

It might be suspected in this experiment that some of the strychnine of the first experiment was not yet absorbed and aided in the result of the second injection. The following experiment is free from such a suspicion:

Rabbit, also 1700 grammes, injected 0.9 mgr., distributed among three extremities. Had a number of tonic and clonic convulsions, the first attack 27 minutes after injection; survived. Five days later, the same animal, injected into one leg 1 mgr. of strychnine. No effect.

In this experiment the single injection was even larger than the divided one. Nevertheless convulsions followed the latter, while the former remained ineffective.

My experiments justify the conclusion that in hypodermic injections the distribution of a certain quantity of poison among several places of the body is more effective than the injection of this quantity in a single dose into one place.

Under the influence of the argument that the larger the quantity, the more effective the filtration and the better the absorption, and in the desire to increase the effectiveness of the distribution by increasing the quantity without increasing the dose, I have attempted to employ greater dilutions of the solution of strychnine, but obtained rather puzzling results. I therefore started out to establish, first, the effect of increasing the bulk without changing the dose in single injections. Here my filtration argument received quite a setback. I shall again illustrate the instructive result by a couple of experiments.

Rabbit, 1300 grammes, injected 0.8 mgr. in a dilution of 1:10,000; no effect. Six days later, the same animal, injected 0.6 mgr. in a

dilution of 1:200. Had convulsions after 11 minutes; survived. Five days later, the same animal, injected 0.9 mgr. in a dilution of 1:20,000. No effect. The same animal again, five days later, injected 0.5 mgr. in a 1 per cent solution; violent tetanus after five minutes and dead. 0.5 mgr. in a 1 per cent solution kills the animal in a few minutes, while nearly twice as strong a dose in a dilution of 1:20,000 remained without any effect.

Guinea-pig, 560 grammes, injected 3.4 mgr. in a dilution of 1:20,000; no effect. Ten days later, injected 2.3 mgr. in a 1 per cent solution. Tetanus and death in a few minutes.

These experiments, which gave uniform results, demonstrate unmistakably that the bulk is nothing and the concentration everything; the doses being equal, the more concentrated the injected poison is, the stronger is the result. The meaning is plain: the osmotic pressure is the most important factor in the process of absorption.

The results of these series of experiments are in brief as follows:

The effect of the subcutaneous injection depends to a considerable degree upon the concentration of the injected solutions, and is materially influenced by a greater distribution of the injected quantity over several areas.

OBSERVATIONS ON A CASE OF CYCLIC ALBUMINURIA.

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In recent years considerable attention has been devoted to the transitory or intermittent appearance of small quantities of proteids in the urine. With the establishment of the fact that the urine of healthy individuals may regularly contain appreciable, though small, amounts of albuminous substances,¹ the significance of so-called "physiological" albuminuria has again been called into question. Ordinarily three general types of "physiological" or "functional" albuminuria have been distinguished, viz., the transitory, intermittent and cyclic. These names indicate with sufficient accuracy the variations in proteid excretion which they are intended to differentiate. The term "cyclic" albuminuria has been applied to those conditions in which the proteid is found in the urine only at approximately regular intervals or at definite periods of the day. In how far the distinctions between these various types are actual remains for future investigation to determine; some observers are at present inclined to regard them merely as different expressions or symptoms of the same condition, rather than as the outcome of typical and specific causes.² The expression "physiological" albuminuria is an undesirable one, since healthy individuals ordinarily fail to show this symptom. There are, however, many other functions in the animal body where the border-line between the physiological and the pathological occasionally becomes obscure; from the clinical standpoint "physiological" albuminuria may for the present be interpreted as applying to conditions where the excretion of proteid has no abnormal significance. The observations recorded in this paper are intended as a contribution to our knowledge of one type of persistent albuminuria.

The subject of these observations first discovered the presence of

¹ K. A. H. Mörner. *Skandinav. Arch. f. Physiologie*, 1895, vi, p. 332.

² C. Flensburg. *Ibid.*, 1893, iv, p. 412.

coagulable proteids in his urine in May, 1899, while a student in the laboratory of physiological chemistry. Since then the excretion of proteid has been studied at various intervals until the present time (February, 1901). During this period no marked change in the quantity eliminated daily has been noted and the general features of the case have remained rather constant. The individual is now 24 years of age, weighs 126 pounds (57.4 kilos), and is 5 feet 7½ inches in height. A very careful physical examination by Professor J. S. Ely failed to indicate any abnormal condition. A recent blood count showing 5,200,000 erythrocytes and 5100 leucocytes per cubic mm. may be taken as typical. Urine samples collected at various times, and particularly during the periods of most pronounced proteid excretion, have been examined after centrifugalization; the collected sediment has not at any time disclosed the presence of unusual structural elements. Furthermore, quantitative determinations of the main urinary constituents have given no evidence of abnormal conditions, as will be seen in the following table:

ANALYSIS OF URINE OF MAY 9, 1900.

Total quantity, 970 cc.; reaction, acid; specific gravity, 1.027

Total Nitrogen	14.39	grammes.
“ P ₂ O ₅	2.13	“
“ S O ₄	2.16	“
Ethereal S O ₄	0.16	“
Chlorine as Na Cl	15.68	“
Proteids	0.29	“
Sugar	absent.	

Methods of Observation.—In making the present observations it has been customary to collect the urine at brief intervals (usually hourly or two-hourly periods) during the waking hours. After noting the physical character (specific gravity, volume, reaction, etc.) of each sample, it was filtered and heated for half an hour or more in a boiling water-bath, a few drops of dilute acetic acid being added, when necessary, to facilitate the coagulation of the proteids. The latter were removed by filtration through weighed, ashless papers, washed free from chlorides and dried to constant weight. It was found impracticable to determine the quantity of proteid by analysis of a fraction of the total daily output as was done in the interesting investigation of Sollmann and McComb,³ since the average daily

³ *Journal of Experimental Medicine*, 1898, iii, p. 137.

excretion of proteid was considerably smaller, and a satisfactory coagulation was almost impossible in the dilute fluid. This objection did not apply, however, to the hourly examinations; for although proteid was absent from the urine at certain periods of the day, the quantity present in intervening periods was correspondingly larger and thus more easily separated. Furthermore, it seemed especially important to study the time relations in the excretion, hence the frequent collection of the urine.

Average Results.—To demonstrate the typical cycle of proteid excretion in this individual, the results of a few analyses made under ordinary conditions of diet and occupation (laboratory work) are appended. They are selected from a number of observations taken at various times and indicate the character and constancy of the albuminuria.

TABLE I.
URINE EXAMINATION.—HOURLY PERIODS.

DATE.	7.15 A. M.		8 A. M.		9 A. M.		10 A. M.		11 A. M.		12 M.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900 ..	473	0	35	0	42	17	45	0	130	0	30	10
16, XI, 1900.	185	0	35	20	44	34	70	36	32	15	35	23
25, I, 1901 ..	181	0	35	32	67	17	123	23	52	40	41	32
Averages ...	283	0	35	18	51	23	79	20	71	18	35	22

DATE.	1 P. M.		2 P. M.		3 P. M.		4 P. M.		5 P. M.		6 P. M.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900 ..	58	0	30	0	47	37	55	0	43	0	69	0
16, XI, 1900.	29	46	35	158	33	137	21	63	28	27	36	18
25, I, 1901 ..	37	25	55	12	42	15	43	17	40	19	36	26
Averages ...	41	24	40	57	41	63	40	27	37	15	47	15

DATE.	7 P. M.		8 P. M.		9 P. M.		10 P. M.		Day's Totals	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900	60	0	23	14	30	29	90	0	1280	107
16, XI, 1900	40	35	45	30	73	0	65	0	806	642
25, I, 1901	51	14	70	4	60	13	82	11	1015	298
Averages	50	16	46	16	54	13	79	4	1034	349

TABLE 1—*Continued.*

URINE EXAMINATION.—TWO-HOURLY PERIODS.

DATE.	7.15 A. M.		8 A. M.		10 A. M.		12 M.		2 P. M.	
	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.
16, III, 1900	195	0	50	25	86	20	62	70	87	43
17, III, 1900	164	14	24	20	83	42	45	76	55	54
21, III, 1900	185	1	70	17	70	22	78	28	62	87
7, VI, 1900	80	0	37	14	232	1	105	14	75	33
Averages	156	4	45	19	118	21	73	49	70	54

DATE.	4 P. M.		6 P. M.		8 P. M.		10 P. M.		Day's Totals.	
	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.
16, III, 1900	85	79	56	35	65	18	123	6	809	298
17, III, 1900	95	21	95	56	108	33	627	6	1294	321
21, III, 1900	57	86	70	35	86	177	71	22	749	474
7, VI, 1900	60	46	70	40	64	27	60	13	783	177
Averages	74	58	73	42	81	64	220	12	909	318

The average daily excretion of coagulable proteid on these 7 days was 331 mgrs. The hourly variations in the quantity excreted correspond quite closely with those observed by previous investigators for this type of albuminuria.⁴ It has been found that while the individual remains in bed, and perhaps during the period immediately after rising, the urine is usually free from coagulable proteid. The excretion then begins, gradually increases in quantity until about noon, when it falls off again; a second but smaller rise occurs later in the afternoon, and towards evening the output is again greatly diminished.

To account for such phenomena various factors have been taken into consideration, such as excessive muscular movements, psychical activity, dietetic influences, and in particular the changes in the posture of the body. There are cases in which each of these influences may apparently be the determining factor. In the present instance an attempt has been made to study the influence of some of

⁴ L. Krehl. *Pathologische Physiologie*, p. 463, Leipzig, 1898.

the conditions referred to, and the results of these observations are detailed below.

Influence of Diet.—In their study of a case of cyclic albuminuria, Sollmann and McComb,⁵ like many previous observers,⁶ failed to detect any changes due to the character of the diet. Our own observations during days in which the food ingested contained a preponderance of either carbohydrate or proteid (meat and eggs), or during fasting, likewise gave no evidence of concomitant variations in the proteid excreted. (Cf. Table II.)

In order to determine still more positively the possible influence of ingestion of food on the excretion of proteid, the following experiment was tried. The subject was awakened from deep sleep at 3 a. m. and ate a rather hearty meal, consisting of bread and butter, milk, chops, marmalade, cakes, etc. The mealtime occupied about 15 minutes, during which the individual was careful not to move from the recumbent position and immediately thereafter he fell asleep again. The urine collected on arising at 7.15 a. m. was entirely free from proteids, corresponding with the experience gained under ordinary conditions. Similar in its bearings on the origin of the excreted proteid is the fact fails to occur regularly during the periods of heightened digestive evident in all the tables that the most pronounced proteid output activity. The total excretion of proteid on the day following this experiment was 355 mgr. in 1168 cc. of urine—figures quite comparable with those obtained on other days.

Influence of Muscular Work.—To ascertain the influence of vigorous muscular exertion, observations were made on days in which strenuous exercise—long walks—was indulged in. No rise in proteid output was observed. Thus, in one experiment recorded in the appended table (Table II, 24, I, 1901), the individual arose at 5 a. m. and took long walks at a brisk pace during various periods of the day, in addition to performing his regular work in the laboratory. Nevertheless the cyclic course of the albuminuria showed no noticeable changes and the day's output of proteid reached a total of only 327 mgr.

⁵ Loc. cit.

⁶ G. Klemperer. *Zeitschr. f. klin. Med.*, 1887, xii, p. 177.

Influence of Posture.—The influence of the posture of the body on the excretion of proteid in cyclic albuminuria of the Pavy type has been especially studied by K. Osswald.⁷ He concludes from observations on three subjects and a study of the records of previous writers that a quiet horizontal posture always diminishes the intensity of the albuminuria; and muscular exercise during the maintenance of this posture fails to incite an excretion of proteid unless an excessive amount of work is done. The upright position, however, is of decided influence, while work done in the sitting posture is without effect. With these observations our own experience in the present instance closely corresponds. Proteid was never present in the urine when the subject arose from bed; but in order to exclude any possible influence of the period of the day aside from personal factors, the individual remained in bed during the daytime and the influence of the horizontal posture was studied when all the other elements were unchanged. The absence of proteids from the urine was repeatedly demonstrated under such conditions and is well illustrated by the protocol of 1, II, 1901 (Table II), where the albuminuria did not begin until the subject arose at 4 p. m.

Nature of the Proteids.—Urine collected during the hours of most pronounced albuminuria showed albumin to be the most conspicuous proteid present; for while we have always obtained a precipitate of proteid (with some urate) when ammonium sulphate was added to saturation, treatment of the urine with saturated ammonium sulphate solution added to half-saturation failed to show even traces of globulin. Nucleo-albumin was not detected even when the urine was acidified after dialysis.⁸ Proteoses were also absent.

SUMMARY.

The preceding observations record a new instance of the occurrence of cyclic albuminuria in an otherwise apparently healthy young man. The typical course of the proteid excretion from hour to hour under

⁷ *Zeitschr. f. klin. Med.*, 1894, xxvi, p. 117. Appended to this paper will be found an extensive list of references to the literature on cyclic albuminuria.

⁸ C. Flensburg. *Skandinav. Arch. f. Physiologie*, 1893, iv, p. 416.

various conditions has been reviewed and its independence of the changes in diet or muscular work pointed out. No relationship between the volume of urine eliminated and the quantity of proteid excreted has been ascertained. The specific effect of the horizontal posture in dispelling the albuminuria is the most interesting feature observed. The attempt to refer this to attendant circulatory changes in the kidneys is, for the present, no more than an interesting speculation.⁹

TABLE II.
URINE EXAMINATION.—EXPERIMENTAL CONDITIONS.

DATE.	REMARKS.	5 A. M.		6 A. M.		7.15 A. M.		8 A. M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	143	0	31	25
5, IV, 1900	Diet largely carbohydrate	111	0	29	26
8, IV, 1900	Subject remained in bed until 5.30 P. M.	143	0	62	0
4, XII, 1900	Diet largely proteid.	193	0	120	1
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	93	0	29	10
23, I, 1901	Additional hearty meal in bed at 3 A. M.	112	0	25	21
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	180	0	52	24	100	10	177	1
1, II, 1901	Subject remained in bed until 4 P. M.	122	0	49	0
DATE.	REMARKS.	9 A. M.		10 A. M.		11 A. M.		12 M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	153	43	20	28
5, IV, 1900	Diet largely carbohydrate	123	9	56	40
8, IV, 1900	Subject remained in bed until 5.30 P. M.	80	13
4, XII, 1900	Diet largely proteid.	204	45	32	5	55	33	36	43
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	102	17	53	44	32	50	46	11
23, I, 1901	Additional hearty meal in bed at 3 A. M.	109	20	183	11	77	32	67	36
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	400	24	211	13	17	29	50	53
1, II, 1901	Subject remained in bed until 4 P. M.	80	1	210	0	260	1	195	0

⁹ A. Charrin. *Journal de Physiologie*, 1901, iii, p. 62.

TABLE II--Continued.

DATE.	REMARKS.	1 P. M.		2 P. M.		3 P. M.		4 P. M.	
		Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.
4, IV, 1900	Diet largely carbohydrate	49	36	54	78
5, IV, 1900	Diet largely carbohydrate	53	42	168	24
8, IV, 1900	Subject remained in bed until 5.30 P. M.	93	13	365	0
4, XII, 1900	Diet largely proteid.	45	60	40	27	116	8	106	15
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	11	14	20	27	163	9	92	16
23, I, 1901	Additional hearty meal in bed at 3 A. M.	25	41	47	26			44	31
24, I, 1901	Arose at 5 A. M. Vigor- ous exercise all day.	50	13	54	25		32	38	41
1, II, 1901	Subject remained in bed until 4 P. M.	166	0	105	1	168	0	127	0

DATE.	REMARKS.	5 P. M.		6 P. M.		7 P. M.		8 P. M.	
		Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.
4, IV, 1900	Diet largely carbohydrate	30	33	115	16
5, IV, 1900	Diet largely carbohydrate	34	74	62	8
8, IV, 1900	Subject remained in bed until 5.30 P. M.	97	1	68	49
4, XII, 1900	Diet largely proteid.	30	18	45	22	65	6	41	6
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	62	4	32	1	28	15	132	7
23, I, 1901	Additional hearty meal in bed at 3 A. M.	85	0	48	22	51	3	81	1
24, I, 1901	Arose at 5 A. M. Vigor- ous exercise all day.	45	26	37	15	52	18	80	5
1, II, 1901	Subject remained in bed until 4 P. M.	40	26	43	13	54	1	49	1

DATE.	REMARKS.	9 P. M.		10 P. M.		Day's Totals.	
		Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.	Vol. cc.	Proteld mgr.
4, IV, 1900	Diet largely carbohydrate	156	11	751	270
5, IV, 1900	Diet largely carbohydrate	141	10	777	235
8, IV, 1900	Subject remained in bed until 5.30 P. M.	219	43	1127	153
4, XII, 1900	Diet largely proteid.	165	1	231	0	1562	288
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	40	24	50	7	985	268
23, I, 1901	Additional hearty meal in bed at 3 A. M.	114	0	37	88	1168	365
24, I, 1901	Arose at 5 A. M. Vigor- ous exercise all day.	91	14	80	7	1738	327
1, II, 1901	Subject remained in bed until 4 P. M.	122	25	147	9	1937	83

INDEX TO VOL. V

A BSCESSSES caused by *Sporothrix Schenckii*, 77
 Acid intoxication of diabetes, 617
 Acid-resisting bacilli, 205
 Agglutination typhoid, colon, hog-cholera and all bacteria, 353
 Agglutination by of intraperitoneal celloidin capsule, 35
 Agglutinins, 353.
 Albumins in normal and antitoxic sera, 47, 67
 Albuminuria, cyclic, 647
 Alloxuric excretion in leucopenia, 541
 Amylaceous bodies and amyloid substance, 111
 Antitoxic diphtheria serum and globulins, 47, 67
 ATKINSON, J. P. See HISS and ATKINSON.
 ATKINSON, J. P. The fractional precipitation of the globulin and albumin of normal horse's serum and diphtheria antitoxic serum and the antitoxic strength of the precipitates, 67

B ACILLI, acid-resisting, 205
 —, capsulated, in gaseous emphysema, 139, 389
Bacillus coli, *B. enteritidis*, *B. faecalis* alcalig., *B. lactis aerog.*, *B. typhi*, etc., agglutinative and other characters, 353
 — icteroides, *Bacillus X*, and hog-cholera bacillus, comparative study, 215
 — mucosus capsulatus in gaseous emphysema, 139, 389
 Bacterial self-purification of streams, 271
 BAILEY, F. R. Studies on the morphology of ganglion cells in the rabbit. 1. The normal nerve cells. 2. Changes in the nerve cells in rabies, 549
 BALDWIN, H. An experimental study of oxaluria with special reference to its fermentative origin, 27
 Bile, influence on metabolism, 513
 Blood serum, albumins and globulins in normal and antitoxic, 47, 67
 Brain, ganglion cells, 21, 549
 —, gas-cysts, 139
 —, hydrocephalus, 195

Broncho-pneumonia with *Streptothrix*, 155
 BURR, C. W. and MCCARTHY, D. J. Acute internal hydrocephalus. A clinical and pathological study, 195

C APILLARIES, nerves of, 493
 CARROLL, J. See REED and CARROLL.
 Celloidin capsules, use and preparation, 635
 Connective tissue, differential stain, 15
 COWIE, D. M. A preliminary report on acid-resisting bacilli with special reference to their occurrence in the lower animals, 205
 Cyclic albuminuria, 647

D ERMATITIS vesiculo-bullosa et gangrenosa, 91
 Diabetes mellitus, acid intoxication, 617
 — —, chronic interstitial pancreatitis, 397
 — —, lesions of islands of Langerhans, 397, 527
 Diphtheria antitoxin and globulins, 47, 67
 Diverticula of the intestine, 333
 DRESBACH, M. On the toxicity of normal urine, 315
 DURHAM, H. E. Some theoretical considerations upon the nature of agglutinins together with further observations upon *Bacillus typhi abdominalis*, *Bacillus enteritidis*, *Bacillus coli communis*, *Bacillus lactis aërogenes*, and some other bacilli of allied character, 353

E LASTIC fibres in tumors, 131
 EWING, J. Malarial parasitology, 429

F ERMENTATION tests for bacteria, 380
 Fibrin, stain for, 18
 FISCHER, M. H. False diverticula of the intestine, 333

G ANGLION cells, normal, 21, 549
 — — — in rabies, 581
 Gangrene, relation of vessels and nerves to, 91
 Gaseous emphysema caused by *B. mucosus capsulatus*, 139, 389
 Glands of the œsophagus, 319
 Glandular secretion, a new theory, 493
 Globulins in normal and diphtheric antitoxic sera, 47, 67

HÆMATOXYLIN, chloride of iron, 18
 —, phosphotungstic acid, 19
 HAMILTON, A. On the presence of new elastic fibres in tumors, 131
 HEKTOEN, L., and PERKINS, C. F. Refractory subcutaneous abscesses caused by *Sporothrix Schenckii*. A new pathogenic fungus, 77
 HERTER, C. A. The acid intoxication of diabetes in its relation to prognosis, 617
 HEWLETT, A. W. The superficial glands of the œsophagus, 319
 HISS, P. H., and ATKINSON, J. P. Serum-globulin and diphtheric antitoxin. A comparative study of the amount of globulin in normal and antitoxic sera and the relation of the globulins to the antitoxic bodies, 47
 Hog-cholera bacillus, 215, 369
 HOOKER, D. See MENDEL and HOOKER.
 HOWARD, W. T. A case of general gaseous emphysema with gas cysts in the brain formed after death and due to *Bacillus mucosus capsulatus*, with a consideration of the gas-producing properties of certain members of this group in the cadavers of animals, 139
 HUTCHISON, R., and MACLEOD, J. J. R. Alloxuric excretion in a case of leucopenia, 541
 Hyaline degeneration of the islands of Langerhans, 419, 527
 Hydrocephalus, acute internal, 195
 Hypodermic injections, 643

INTESTINAL diverticula, 333
 Iron hæmatoxylin, 18
 Islands of Langerhans in diabetes, 397, 527

JORDAN, E. O. Some observations upon the bacterial self-purification of streams, 271
 JOSLIN, E. P. The influence of bile on metabolism, 513

LARKIN, J. H. See NORRIS and LARKIN.
 Leucopenia, alloxuric excretion, 541
 Lymph-formation, a new theory, 493

MCCARTHY, D. J. See BURR and MCCARTHY.
 MCCRAE, J. Notes upon the agglutinations obtained by intraperitoneal insertion of celloidin capsules containing bacilli and upon a mode of preparing such capsules, 635
 MACLEOD, J. J. R. See HUTCHISON and MACLEOD.
 Malarial parasitology, 429
 MALLORY, F. B. A contribution to staining methods. 1. A differential stain for connective tissue fibrillæ and reticulum. 2. Chloride of iron hæmatoxylin for nuclei and fibrin. 3. Phosphotungstic acid hæmatoxylin for neuroglia fibres, 15
 —. Proliferation and phagocytosis, 1
 MELTZER, S. J. Some experimental data on the significance of concentration and of multiplicity of area in hypodermic injections, 643
 MENDEL, L. B., and HOOKER, D. B. Observations on a case of cyclic albuminuria, 647
 Metabolism, influence of bile, 513
 Milk-whey for cultures, 378
 Muscle, nerve-endings, 493

NERVE cells, normal, 21, 549
 — — —, in rabies, 581
 Nerves of capillaries and muscle, 493
 Neurofibrils in ganglion cells, 21
 Neuroglia, stain for, 19
 NORRIS, C., and LARKIN, J. H. Two cases of necrotic broncho-pneumonia with *Streptothrix*, 155

ÖESOPHAGUS, glands, 319
 ÖRNHÜLS, W. Amyloid substance and amylaceous bodies in multiple syphilitic tumors of the bones with remarks on the relation of amylaceous bodies to amyloid substance, 111
 OPIE, E. L. On the relation of chronic interstitial pancreatitis to the islands Langerhans and to diabetes mellitus, 397
 —. The relation of diabetes mellitus to lesions of the pancreas. Hyaline degeneration of the islands of Langerhans, 527
 Oxaluria, 513

PANCREAS, hyaline degeneration, 419, 527

—, lesions in diabetes, 397, 527

Pancreatitis, chronic interstitial, 397

Parasites of malaria, 429

PATON, S. A study of the neurofibrils in the ganglion cells of the cerebral cortex, 21

PERKINS, C. F. See HEKTOEN and PERKINS.

PERKINS, R. G. Report of a laboratory epizootic among guinea-pigs associated with gaseous emphysema of the liver, spleen and kidneys due to *Bacillus mucosus capsulatus*, 389

Phagocytosis, 1

Phosphotungstic acid hæmatoxylin, 19

Proliferation and phagocytosis, 1

RABIES, nervous lesions, 581

Raynaud's disease, vessels and nerves in, 91

REED, W., and CARROLL, J. A comparative study of the biological characters and pathogenesis of *Bacillus X* (Sternberg), *Bacillus icteroides* (Sanarelli), and the hog-cholera bacillus (Salmon and Smith), 215

Reticulum, stain for, 15

SECRETION, a new theory, 493

Self-purification of steams, 271

Sera, normal and antitoxic, 47, 67

SHILLER, C. The nerves of the capillaries with remarks on nerve-endings in muscle. A new theory of lymph-formation and of glandular secretion, 493

Smegma bacilli, 205

SPILLER, W. G. Pathological report on a case of dermatitis vesiculo-bullosa et gangrænosa mutilans manuum (Duhring), with a consideration of the relations of vascular and nervous changes to spontaneous gangrene and Raynaud's disease, 91

Sporothrix Schenckii, 77

Staining methods (MALLORY), 15

Streptothrix in broncho-pneumonia, 155

Sugars for cultural tests, 380

TOXICITY of urine, 315

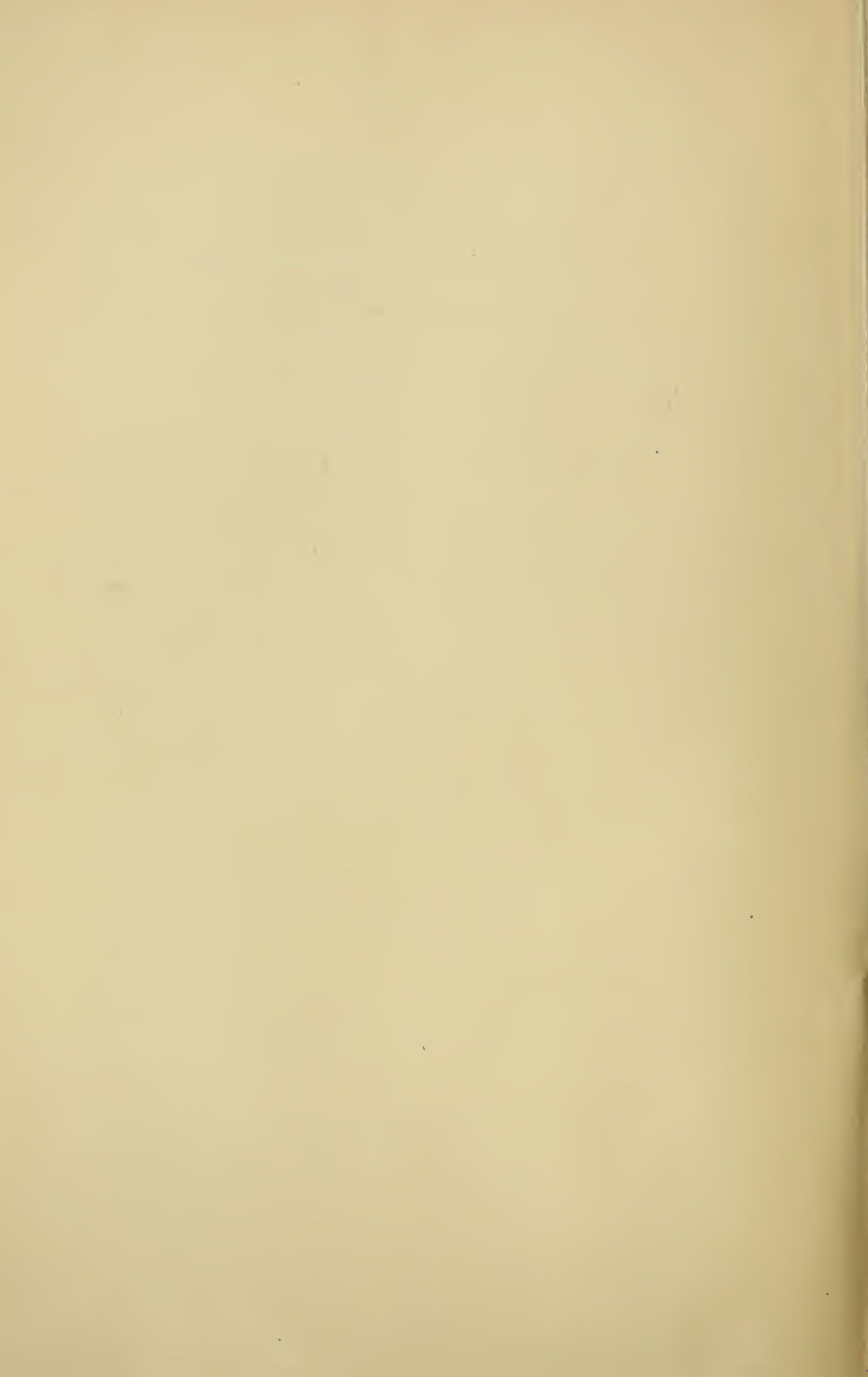
Toxins, effects on cells, 1

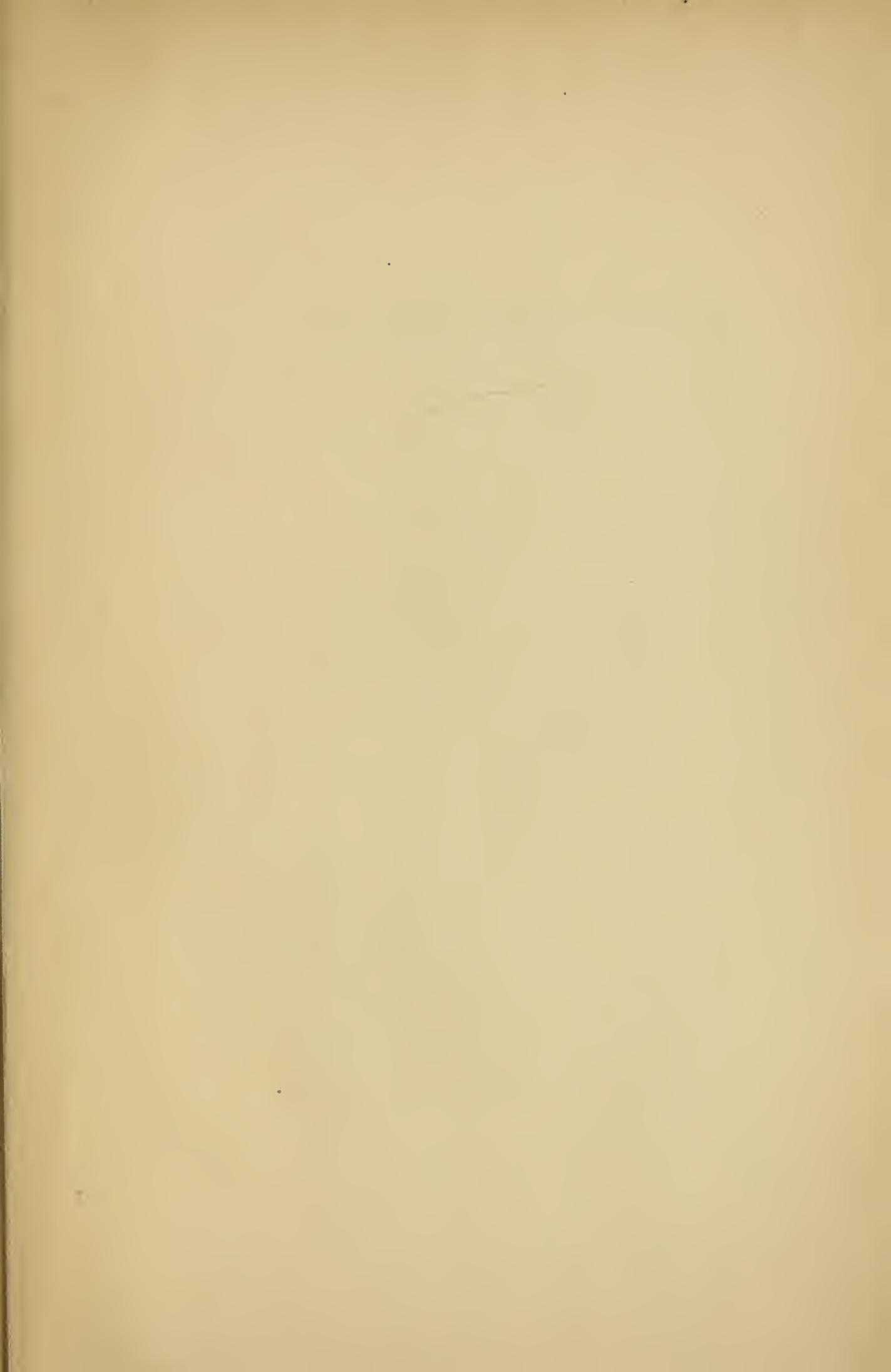
Tumors, elastic fibres in, 131

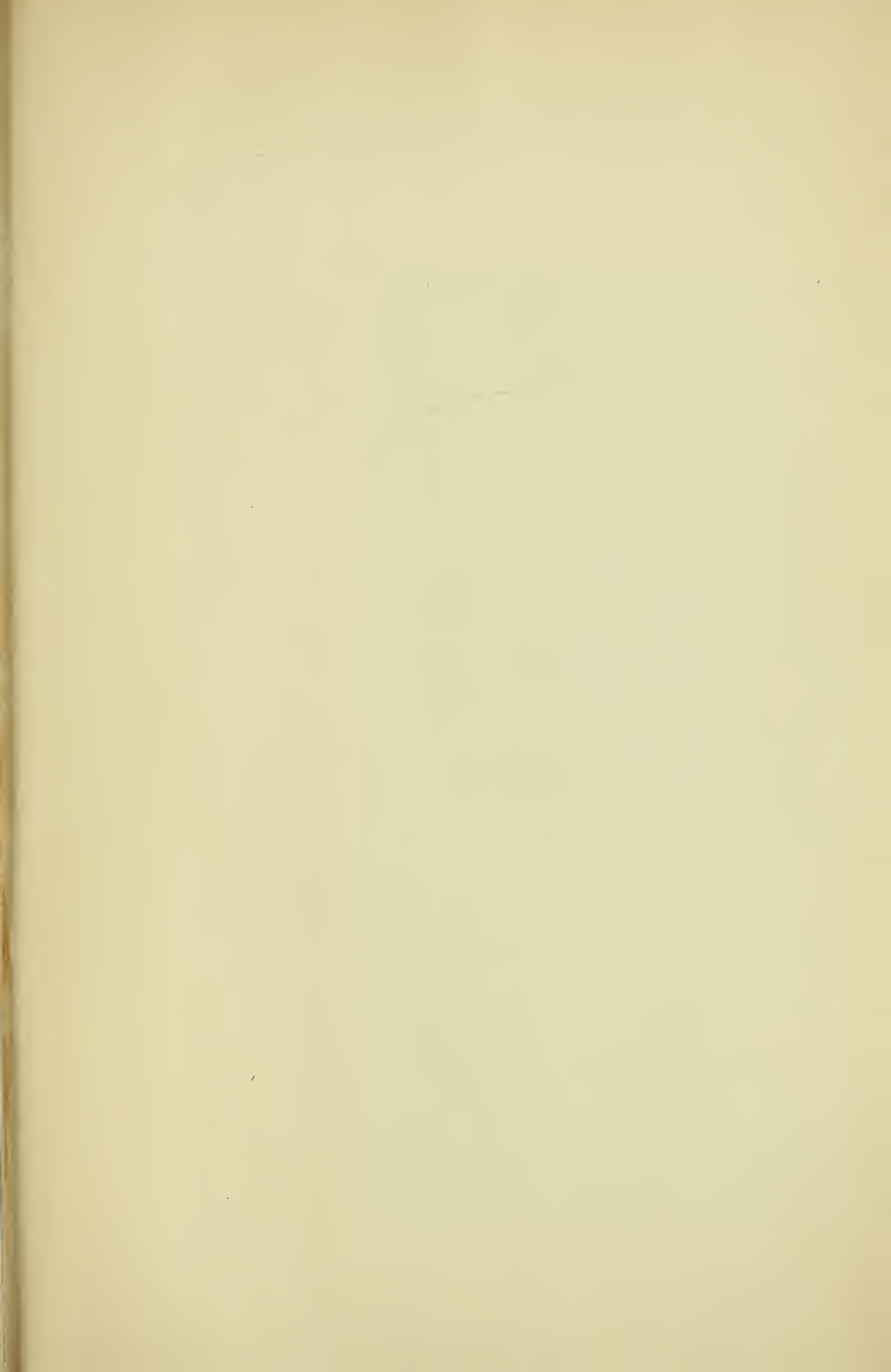
URINE, oxaluria, 27

—, toxicity, 315

WATER, self-purification of, 271







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